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Title:
The effect of veterinary endectocides on the reproductive physiology and output of temperate dung beetle species

The effect of veterinary endectocides on the reproductive physiology and output of temperate dung beetle species

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A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of MSc(Res) in the Faculty of Science, School of Biological Sciences.

Date: 20th August 2018

Word count: 19,284

Abstract

Dung-colonising beetles, commonly known as dung beetles, provide several ecosystem services for farmland pasture systems. However, dung beetles are in decline in northern temperate regions, in part due to widespread endectocide use for control of pests and parasites of cattle. Endectocide residues are excreted in the dung of cattle, at concentrations that are still toxic to insects.

The overall aim of this study was to examine the effects of endectocide exposure on the reproductive output of dung-colonising beetles. To achieve this, the first objective was an examination of the short-term sublethal effects of ivermectin, a macrocyclic lactone, on two common northern temperate species, *Onthophagus similis* (Scriba) and *Aphodius prodromus* (Brahm). Constant exposure of field-collected adult beetles, over a period of 3 weeks, resulted in smaller oocytes of *O. similis* at 1 ppm (wet weight). Beetles also had smaller fat bodies and motility was reduced. The study of *A. prodromus* was inconclusive due to extensive mortality in all treatments.

The second objective was an examination of different long-term endectocide treatment regimes (macrocyclic lactones, synthetic pyrethroids, or no treatment) on 24 beef cattle farms, using pitfall trapped beetle samples. Four species of dung beetle were considered: *Aphodius rufipes* (L.), *Aphodius fossor* (L.), *Onthophagus coenobita* (Herbst) and *O. similis*. Endectocide use was associated with a lower proportion of gravid *O. similis* females. A more variable size of *O. similis* and a smaller size of A. *fossor* was seen on farms using macrocyclic lactones. On the other hand, *A. rufipes* was larger on farms using endectocides, possibly through reduced competition with other species. The reproduction of *A. fossor* or *O. coenobita* did not appear to differ between regimes.

The study suggests that beetle reproductive output is affected by endectocide use and in the long-term, such effects may be as ecologically damaging as lethal effects.

Acknowledgements

I would like to express my gratitude to Dr. Bryony Sands for giving me access to her collection of dung beetles from the field, guidance on trap design and for her knowledge on other aspects of the project. Thank you also to Katie Bell and Abby Parravani for assistance setting up the traps for the experiment in Chapter 2 and to the Amos family for use of their farm. I would also like to express my thanks to Professor Richard Wall for his advice and invaluable guidance throughout this year. Lastly, thank you to Bridget and Roger Weaving for their financial assistance during this year.

Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University of Bristol's Regulations and code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED……..DATE……..

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Chapter 1 – The biology and reproductive systems of dung-colonising beetles

1.1 – Introduction to dung beetles

The beetles that colonise dung, commonly known as dung beetles, are a polyphyletic functional assemblage belonging to the superfamily Scarabaeoidea, comprising of the subfamilies Aphodiinae, Scarabaeinae and Geotrupinae (Hanski & Cambefort, 1991). Dung beetles are defined by their feeding habit of coprophagy, taking advantage of the nutrient-rich liquid found in dung (Holter, 2016). Soft, filtering mandibles are believed to have evolved from hard, biting mandibles to exploit this resource. Most dung beetles are generalist feeders, consuming large herbivore or omnivore dung, but carnivore and small mammal dung are also utilised by some species (Hanski & Cambefort, 1991; Simmons & Ridsdill-Smith, 2011).

Dung beetles can additionally be organised by their oviposition behaviours into three groups: endocoprids, paracoprids and telocoprids (Fig. 1.1). Endocoprid

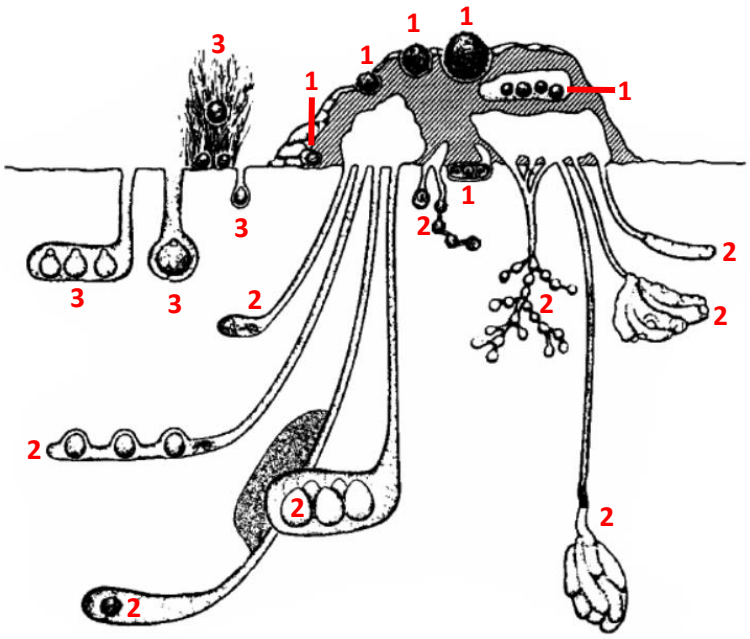

Figure 1.1 Oviposition behaviours of dung beetle species belonging to 3 distinct groups: 1. Endocoprids, 2. Paracoprids, 3. Telocoprids. Adapted from: Floate, 2011.

beetles, comprising mostly of Aphodiinae, remain within the dung or the top layer of soil to lay their eggs, typically not producing an egg chamber. Paracoprid beetles dig tunnels beneath the dung, creating brood chambers. Eggs are contained within brood balls inside the chamber. Geotrupinae are exclusively paracoprid, along with some species of Scarabaeinae, and a few Aphodiines. The final group, the telocoprids, are rolling dung beetles. Telocoprid beetles create dung balls which are rolled away from the dung source and buried, again in brood chambers. The remaining Scarabaeinae belong to this group. Northern temperate regions, the focus of this study, are characterised by the absence of telocoprid beetles. The absence of rolling species allows dung to remain above ground for longer. This leads to an unusually diverse succession of species. Diptera are the first to arrive, such as the yellow dung flies, *Scatophaga stercoraria* (L.), peaking in abundance around day 7 (Lee & Wall, 2006). Dung beetles arrive afterwards, becoming most numerous between days 1 and 4, and declining as a crust is formed on the surface of the dung pat. Diptera and Coleoptera carry phoretic mites and nematodes, which peak in abundance on approximately day 10. After day 10, parasitic wasps and flies visit the pat. The presence of annelid worms is the final stage of succession (Floate *et al*., 2005). In the U.K., seven genera of dung beetles are present, the most numerous being *Aphodius, Onthophagus* and *Geotrupes*. This study focuses on the former two genera.

1.1.1 Aphodius

Aphodiines are small beetles which are largely distributed in northern temperate regions but are also abundant in the tropics and subtropics. Most are endocoprids, laying their eggs in the dung or the soil (Fig. 1.1). All species have a pair of ovaries, but ovariole number varies from 4 to 11 per ovary depending on species (Fig. 1.2; Gittings & Giller, 1997). Lifetime fecundity is thought to be approximately 100 eggs per female and species are typically univoltine (one generation per year) (Hanski & Cambefort, 1991). The whole lifecycle of endocoprid *Aphodius* beetles occurs in dung; larvae feed within the dung, pupate in the dung or soil surface and emerge as adults. This study concentrates on three species of *Aphodius*: *Aphodius rufipes* (L.)*, Aphodius fossor* (L.) and *Aphodius prodromus* (Brahm).

Aphodius rufipes is one of the larger species of *Aphodius* in the U.K. Females develop eggs simultaneously, so will oviposit 6 to 10 eggs per batch (Gittings & Giller, 1997). Adults are most abundant during the late Summer, their earliest flight periods occurring around the middle of May. Emergence is not highly synchronised, so immature beetles may be present throughout the whole Summer (Gittings, 1994). Most individuals will overwinter as final stage larva.

Aphodius fossor is also a relatively large species. Individuals develop eggs sequentially, so eggs will be at several stages of development at any time. Gittings and Giller (1997) found an average of 2 mature eggs per adult female. Eggs are laid singly within the dung pat crust. Overwintering occurs mostly at the adult stage and adults appear mostly in the late Summer. However, again, adult emergence is not well synchronised, so immature beetles may be present throughout the Summer.

Finally, *A. prodromus* is a relatively small species of Aphodiine, ranging from 4 to 6 mm. This species has accumulated sequential development; as eggs mature, they are accumulated, and up to 23 mature eggs can be stored at a time. Each individual has 10 ovarioles in total. Mature eggs are laid in the soil. This species does not breed in dung, preferring decaying plant material, which larvae feed upon. Overwintering occurs as a mature adult, and individuals emerge in the early Summer (Gittings & Giller, 1997). It is thought that this species has higher synchrony of generations than the other *Aphodius* species described. All three species are highly abundant in the U.K.

1.1.2 Onthophagus

Onthophagus are less specious and are of lower population abundance than *Aphodius* in northern temperate regions. Species of this genus are paracoprids; they dig tunnels to bury brood balls below the dung resource. All species of Scarabaeinae, including *Onthophagus*, have only one functional ovary with a single ovariole (Robertson, 1961). This allows only one mature oocyte to be developed at a time, the others being at different, sequential stages of development further down the coiled germarium (Fig. 1.2). *Onthophagus* build shallow egg chambers, with several brood masses. There is no cooperation between the male and female and no maternal care, as demonstrated by some species of Scarabaeinae (Palestrini

& Rolando, 2001). In comparison to other Scarabaeinae, fecundity is thought to be relatively high. Most *Onthophagus* species live for only a single season.

The current study focuses on two species of *Onthophagus*: *Onthophagus coenobita* (Herbst) and *Onthophagus similis* (Scriba). These species are also abundant in the U.K.

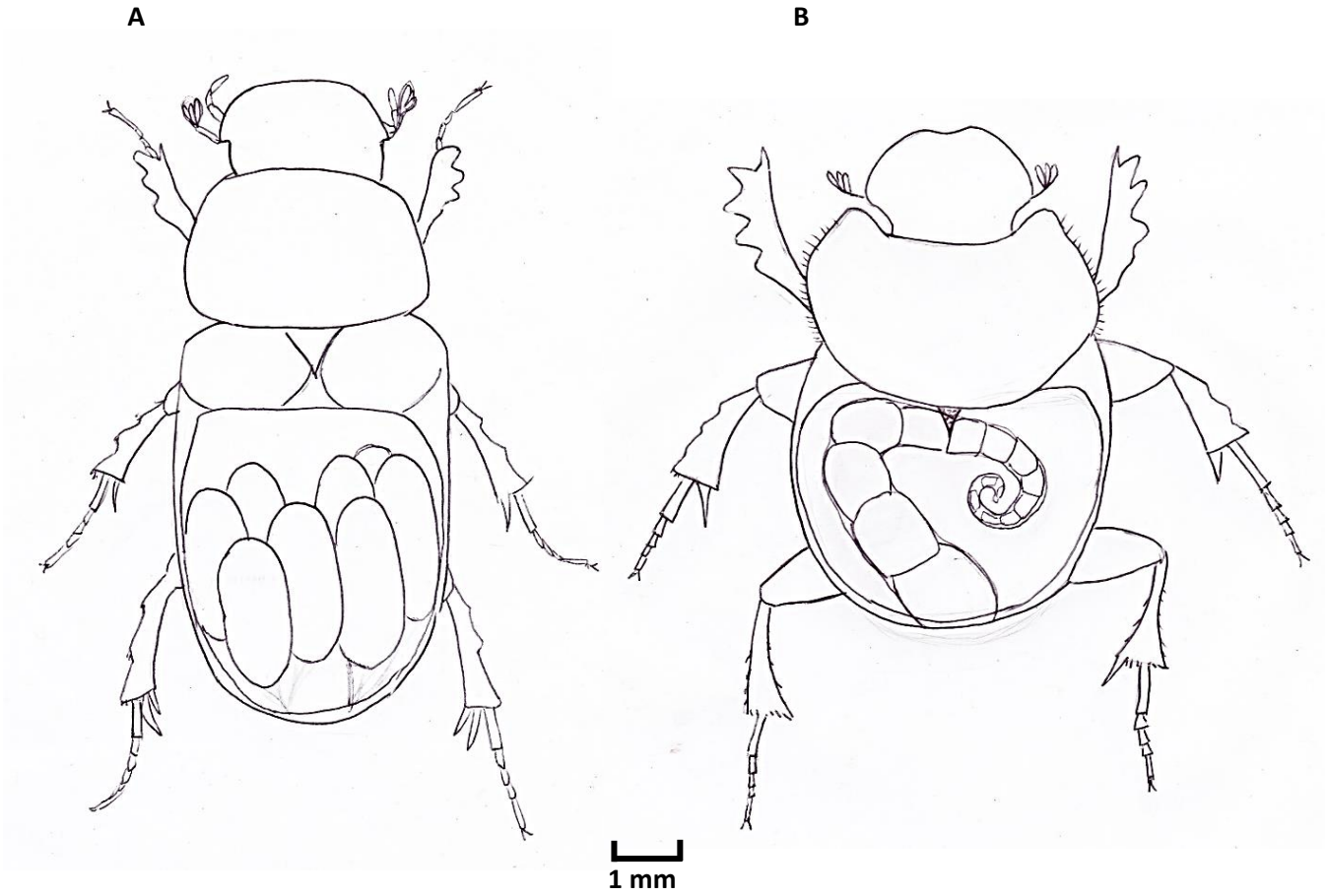

Figure 1.2 Ovaries of A. *Aphodius rufipes* and B. *Onthophagus coenobita* dung beetle species. Drawn from a dissecting microscope.

1.2 - Importance of dung beetles

Insects often play important ecological roles and provide essential ecosystem services. For example, insects act as pollinators, detritivores and regulators of pest abundance (Losey & Vaughan, 2006). Dung beetles are no exception; by removing dung from pastures they provide a suite of services which save the U.K. agricultural industry an estimated £367 million pounds every year (Beynon *et al.,* 2015).

1.2.1 Pasture fouling

The rapid degradation of dung is crucial for cattle farming due to pasture fouling. While dung is still on the surface, the pasture beneath and surrounding the pat is lost as grazing for cattle. Fincher (1981) predicted for every undegraded dung pat an area of pasture five times that of the pat is lost. This is due to avoidance of pasture beneath and surrounding the pat by cattle. When herbage intake by cattle was measured in a study, consumption was found to be 5-10 % lower when pasture was fouled (Spörndly, 2009). Therefore, to optimise grazing productivity, fouling should be reduced.

Dung beetles decrease pasture fouling by increasing rates of dung degradation. They achieve this through tunnelling, which increases the pat surface area available for decomposition by bacteria and fungi. Degradation of dung is retarded when the abundance of dung fauna in dung pats is reduced (Anderson *et al*., 1984; Wall & Strong, 1987; Sommer & Bibby, 2002). Experimentally, removal or exclusion of dung-colonising beetles often results in delayed degradation of dung (Madsen *et al*., 1990; Wardhaugh & Mahon, 1991).

Dung degradation is also affected by a number of other factors, and these cause discrepancies between studies on degradation rates. For example, cattle fed on lush grass will drop dung of a higher water content. A high water content increases the surface area for decomposition, resulting in shorter decomposition times. Additionally, if pasture is of high quality stocking rate is likely to be higher, increasing trampling by cattle and thereby increasing degradation rates. Lastly, season, climate, location and stochastic processes affect abundance of insect colonisers (Hanski & Cambefort, 1991; Floate *et al*., 2005).

1.2.2 Reduction of volatilisation

If dung remains on the pasture valuable nutrients remain trapped within its contents or are lost via volatilisation. Volatilisation is the process by which nutrients, such as nitrogen and ammonia, are released in to the atmosphere as gas. It has been estimated that 22 - 36% of nitrogen is lost from cattle waste by volatilisation (Smil, 1999). As a result, farmers must replace nitrogen in soils using fertiliser to improve yields. A 2006 FAO report highlighted that humans have doubled the amount of global nitrogen entering the nitrogen cycle using the Haber-Bosch process (Steinfeld *et al*., 2006). This increase can contribute to environmental issues, such as pollution of water sources by leeching.

By the processes of tunnelling, dung beetle species mix dung with soil, preventing loss by volatilisation; increases in carbon, potassium, nitrogen, calcium and magnesium have been found in the soil beneath dung pats in laboratory experiments (Kazuhira *et al*., 1991; Yokoyama *et al.*, 1991; Bertone, 2004; Yamada *et al.,* 2007). Losey and Vaughan (2006) predicted if dung beetles were absent from the US that an additional 131 million kg of nitrogen would be lost.

As nitrogen can be a limiting nutrient in pasture systems, its availability may affect plant productivity. Bang *et al.* (2005) looked at the biomass of herbage after treatment with 3 species of telocoprid dung beetles in comparison to plots which excluded dung beetles. After 3 months, biomass of herbage and its nitrogen content was greater in the treatment group than the control group. Dry herbage biomass increased by 17% on average over 3 months. Nitrogen content had increased in the soil by approximately 0.5% at the end of the experiment. Furthermore, Southcott (1980) noted that when the anthelmintic phenothiazine was used in sheep, which eliminates dung beetles, there was a reduction in herbage and nitrification rates in comparison to the control. The study fails to quantify herbage and nitrogen reductions. However, it does state that after four years of the experiment the average body weight of ewes was reduced by 7.7 kg, when sheep were at a high stocking rate.

Additionally, tunnelling creates aerobic conditions in the dung pat (Kazuhira *et al*., 1991). This alters the bacterial fauna in the pat and soil (Yokoyama *et al*., 1991). Bacterial fauna convert nutrients into useable forms for plants. Therefore,

alteration in the fauna could affect the amount of nutrients available. Little is known of the effect of dung beetles on bacterial fauna, so this is an area suitable for future research.

1.2.3 Reduction of greenhouse gas emissions

Agriculture is responsible for approximately 30% of greenhouse emissions, with livestock contributing approximately two-thirds of these emissions (Tubiello *et al.*, 2013). Dung pats left on fields for extended periods are a source of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Burial of dung below ground by tunnelling should decrease the amount of greenhouse gases emitted. Additionally, the generation of aerobic conditions in the pat should reduce the production of compounds formed under anaerobic conditions. For example, methane is produced via methanogenesis under these conditions. However, the various studies conducted have produced conflicting results.

In the first study of its kind, closed chambers were used in Finland to measure emissions from pats with or without a mixed assemblage of *Aphodius* dung beetles (Penttila *et al*., 2013). Over the Summer grazing period it was found that in the presence of beetles, total emissions of CH_4 were 39% lower, but N_2O emissions were higher. There were negligible differences in the amount of CO_2 emitted. This equated to a 0.6% reduction of CO_2 equivalents. Additional studies have also used a closed chamber method. A further study in Finland found a reduction of 7% in CO_2 equivalents in the presence of a mixed dung beetle assemblage, this time including a species of *Geotrupes* (Slade *et al*., 2016). However, other studies have found increases in greenhouse gases in the presence of dung beetles. Iwasa *et al.* (2015) examined the impact of the beetle *Caccobius jessoensis* (Harold), and found a 370% increase in CO_2 and a 58% reduction in CH_4, with no difference in N_2O. The conflicting results between studies are likely to be due to the different assemblages of beetles used in the studies. Indeed, Piccini *et al*. (2017) found species contribute unevenly to greenhouse emissions; greater reductions were achieved by using multiple species mixes, providing a 32% reduction of CO_2 equivalents, including reductions in N_2O emissions. Additionally, studies vary in length; the Iwasa *et al.* (2015) study was only a week long, whereas other studies have extended for up to an entire grazing season. As emissions are irregular, measuring fluctuations for the whole season is likely to be preferable (Penttila *et al*., 2013).

However, emissions should be considered over the entire production system, as opposed to just the pasture level. In this context, Slade *et al.* (2016) estimated that in Finland the presence of dung beetles resulted in only a 0.08% reduction in greenhouse gas emissions in the milk industry and 0.13% in the beef industry. Slade *et al*. therefore argue that emissions should be reduced in other ways, such as energy use in the sector to produce a greater impact. However, in part, this conclusion was due to relatively little dung deposited on pastures in Finland, as the grazing season is roughly 100 days. For the remainder of the season cattle are indoors. In other regions cattle spend the whole year outside. Therefore, in these regions the effect of dung beetles on greenhouse gases emissions is likely to be greater.

1.2.4 Removal of breeding sites

Ectoparasites of cattle, including flies, ticks, mites and lice, are of great economic importance in the cattle industry. In the U.S. losses due to ectoparasites are predicted to exceed 2.2 billion dollars per year, excluding the costs of endectocides as a means of control (Byford *et al*., 1992). Some of these ectoparasites are reliant on cattle dung for feeding or breeding, some of the most important of these are horn flies (*Haematobia irritans* (L.)), face flies (*Musca autumnalis* (De Geer)) and bush flies (*Musca vetustussima* (Walker)).

Horn flies are obligate blood sucking parasites of cattle; eggs are deposited in cattle dung (Foil & Hogsette, 1994). Of all the cattle ectoparasites found in the U.S., horn flies are thought to be of the greatest economic importance. Horn flies are responsible for reduced weight gain due to irritation, resulting in reduced feeding rates (Duren, 1975; Harvey & Launchbaugh, 1982). Campbell (1976) found pesticide dust bags on cattle, used to kill flies, resulted in a 6 kg greater weight gain in the control group without bags. Depending on study and year, a weight gain of 2.8% to 17.7% was found by ear tagging to control flies (Haufe, 1982; Kunz *et al*., 1984). Drummond (1987) reviewed the data from many studies and found a correlation between infestation level and loss of average daily weight gain. Additionally, horn flies can transmit the filarial nematode, *Stephanofilaria stilesi* (Foil & Hogsette, 1994). This nematode causes skin lesions in cattle.

Face and bush flies feed on secretions from the eyes of cattle and eggs are laid in dung (Krafsur & Moon, 1997). Face flies cause negligible losses to meat and milk yield but spread the disease, bovine keratoconjunctivitis, known as pinkeye, caused by the bacterium, *Moraxella bovis*. Infected animals must be removed from the herd, monitored and treated with antibiotics. Untreated animals can develop blindness. There are currently no vaccines to prevent the disease (Brown *et al*., 1998). Bush flies also cause irritation to cattle, as well as people.

Dung beetles aerate dung by tunnelling, creating a desiccating environment which increases mortality of Dipteran larvae and eggs (Walsh & Cordo, 1997). This is due to the dependence of eggs and larvae on moisture for survival (Hughes *et al*., 1972). During field observations when abundance of dung beetles is higher, fewer flies emerge from dung pats (Walsh & Cordo, 1997). Another field study looked at bushfly populations in Australia over a period of 8 years, with dung beetles introduced after 5 years (Tyndale-Biscoe & Vogt, 1996). After dung beetles were introduced, the study found a significant reduction of nulliparous female flies, indicating that the beetles were contributing their control.

This effect of dung beetles on dung-breeding Diptera is more pronounced in laboratory trials. Hughes *et al*. (1979) found that beetles were successful at supressing bushfly numbers. At the abundance of 200 beetles per dung pat the number of flies that successfully pupated was reduced to below 10%. Similarly, the beetle *Onthophagus gazelle* (Fabricius) was found to reduce numbers of bush fly by 80 to 100% (Bornemissza, 1970). The clear effects of beetles seen in laboratory studies may be due to the simplified ecosystem and the higher abundance of starved beetles compared to natural conditions.

1.2.5 Control of gastrointestinal parasites

Helminth worms cause economic losses in the cattle industry through parasitism. Adult worms are found in the abomasum, small intestine or large intestine (Sutherland & Leathwick, 2011). Eggs are shed into the faeces and hatch as larvae. The larvae then go through several moults, developing in to infective 3rd stage larvae (L3s). L3s move on to herbage surrounding the pat and wait to be ingested by cattle. When cattle are infected by helminths reductions in feed intake, weight,

yield of meat or milk and fecundity are seen (Perry & Randolf, 1999). Anthelmintics are used to keep parasites at acceptable levels so yield losses are at a minimum.

Nichols and Gomez (2014) reported that dung beetles come in to contact with at least 19 families of Platyhelminths (flatworms) and Nematoda (nematodes), some of these species being parasites of cattle. A few species even use dung beetles as an intermediate host (Gottlieb *et al*., 2011). Therefore, there is potential to alter interactions between cattle and their parasites at the egg or larval stage.

It has been suggested that dung beetles can reduce the number of viable helminth eggs by destroying them with their mouthparts. In general, however, larger beetle species destroy a greater number of eggs than smaller species, since the smaller beetle species filter out helminth eggs without damaging them (Holter *et al*., 2002). For example, the relatively large *Geotrupes stercorosus* (L.) destroyed 50% of eggs (Bílý & Prokopič, 1977). In comparison, Holter (2000) found six species of *Aphodius* could only ingest particles of between 5 and 25 µm diameter. Since *Ascaris* eggs are approximately 70 x 50 µm in diameter and strongyle eggs are 90 x 40 µm, they are unlikely to reach the mandibles (Bíly & Prokopic, 1977; Cuomo *et al*., 2012). However, another study found evidence of *Aphodius* species destroying *Cryptosporidium parvum* eggs, a species which causes cryptosporidiosis (Mathison and Ditrich, 1999). These eggs are much smaller, at 4 – 6 µm in diameter. Despite the success of relatively large species at crushing eggs, an additional study found some damaged eggs to still be viable after incubation (Miller *et al*., 1961).

Additionally, the burying of brood balls by paracoprid and telocoprid dung beetles can reduce abundance of L3s. Larger species of beetle are likely to be superior in reducing larval counts as they bury brood balls deeper. If the maximum vertical migration distance of larvae is not far enough they may await favourable conditions and emerge later (Gregory *et al*., 2015).

English (1979) placed pats with known faecal egg count (FEC) on pasture. At peak *O. gazelle* activity, in February and March, there were reductions of 60% in the number of L3 nematodes found in the surrounding herbage. In another experiment, initially a greater number of L3s were found on herbage surrounding pats which included dung beetles. However, after 8 weeks of the experiment, pats with dung beetles had lower numbers of L3s (Sands *et al*., 2017). Egg hatching is dependent

on availability of oxygen so the aeration by dung beetles could initially mean greater numbers of eggs hatch (Nielsen *et al.*, 2010). Fincher (1973) also found that where dung beetles were absent, egg and larval counts of the nematode *Ostertagia ostertagi* were highest.

1.2.6 Food web

Lastly, the dung beetle guild forms part of a complex food web. For example, larvae and adult dung beetles are a food source for bats and birds. This includes the protected greater horseshoe bat, *Rhinolophus ferrumequinum*, of which there are only approximately 4,000 individuals in the U.K. (DEFRA, 2013).

1.2.7 Conclusion

Beynon *et al*. (2015) estimated the value of the ecosystem services dung beetles provide in the U.K. to be £368 million per year. There is some evidence that this may be an over-estimate as Sands *et al.* (2017) calculated a 19% increase of nematode L3s when dung beetles were excluded, which is substantially less than the value used in Beynon's study. However, the figure still is useful to give an idea of the importance of dung beetles in pasture systems and may help give dung beetles higher priority of conservation (Losey & Vaughan, 2006). Others argue that placing economic value on the natural world is inappropriate as it allows equivalence between natural and man-made provisions of ecosystem functions (McCauley, 2006). In this view it is also important to view dung beetles as part of a balanced, well-functioning ecosystem on which other organisms rely.

1.3 - Introduction to veterinary endectocides

Before the discovery of synthetic pesticides and parasiticides, the use of chemicals for pest and parasite control was limited in agriculture. As a result, pests and parasites were major constraints on the productivity of livestock. The pesticide revolution came in the late 1930s when Paul Muller rediscovered DDT (Dichlorodiphenyltrichloroethane). It became globally used, increasing yields and decreasing prevalence of diseases such as typhoid and malaria. Following the introduction of DDT many other pesticides were developed. This began with additional organochlorines and cyclodienes in the 1940s, followed by

organophosphates in the 1950s, and pyrethroids in the 1970s. Macrocyclic lactones (MLs), the main focus of this study, were developed in the 1980s. Finally, neonicotinoids, which include imidacloprid, the most widely used pesticide in the world, were developed in the 1990s, although these are not currently used in livestock treatment (Casida & Quistad, 1998). Cattle farming in the U.K. uses MLs as the predominant approach to pest and parasite control: a survey of beef and dairy farms in the South West England found that 67% used MLs for endoparasite and ectoparasite control (Stafford & Coles, 1999). The discovery of this class of compounds was important due to their efficacy against both endoparasites and ectoparasites. Synthetic pyrethroids (SPs), the other focus of this study, are commonly used for treatment of endoparasites in organic farming (Soil Association, 2018).

1.3.1 Macrocyclic lactones

The macrocyclic lactones are divided into the avermectins (including ivermectin, eprinomectin and doramectin), the milbemycins (moxidectin) and the recently discovered spinosyns. They are broad spectrum endectocides, active against nematodes, lungworms, warbles, mites, lice, round worms and gastrointestinal worms (Floate *et al.*, 2005). The avermectins are derived from streptomycetes gram positive bacteria (Burg *et al*., 1979). Ivermectin is the most widely used avermectin and is the focus of much of this study (Campbell *et al*., 1989). Ivermectin (22,23-dihydroavermectin B_1) was introduced in 1981 and is a synthetic derivative of abamectin. It comprises at least 80% 22,23-dihydroavermectin B_{1a} and no more than 20% 22,23-dihydroavermectin B_{1b} (Sutherland & Campbell, 1990). In addition to its use as a veterinary endectocide, it is also used in human medicine as a microfilaricide for *Onchocerca volvulus* which causes onchocerciasis (Taylor *et al*., 1990).

Avermectins act by interfering with invertebrate neurotransmission (Chabala *et al*., 1980). They increase the permeability of membranes to chloride ions due to agonistic action of the compound on chloride channels. The influx of chloride ions reduces the frequency of action potentials. Ivermectin works on GABA (γ–aminobutyric acid) gated and glutamate-gated chloride channels (Putter *et al*., 1981). These channels are abundant in many pest and parasite species. The reduced frequency of action potentials can cause paralysis at high concentrations (Zufall *et*

al., 1989). At lower concentrations reduced mobility and partial paralysis is caused. For example, studies have shown the pharynx may be paralysed (Geary *et al.,* 1993). Blockage of the oesophagus prevents ingestion, so organisms die of starvation or desiccation. Additionally, GABA receptors have been found in olfactory sensory neurones. This indicates that ivermectin could also be effective in altering the processes such as the recognition of pheromone signals (Pregitzer *et al*., 2013).

Administration of macrocyclic lactones to cattle can be via subcutaneous or intramuscular injection, topically (pour-on), orally, by ear-tag or via a sustained-release bolus mechanism, which is lodged in the rumen of the animal. Due to low solubility in water, in mammalian hosts the compound is found mainly in plasma and adipose tissue, such as fat and liver tissue (Schnitzerling & Nolan, 1985). Vertebrates lack GluCl entirely and GABA gated chloride channels are only found in the central nervous system. As the compound has difficultly crossing the blood-brain barrier, tests indicate a wide margin of safety for the compound in mammals. For example, it was found a rat brain had a 100-fold lower affinity for ivermectin than the brain of *Caenorhabditis elegans* (Schaeffer & Haines, 1989).

Once metabolised in the body, up to 98% of the administered compound is found in the faeces (Sommer *et al*., 1992). Halley *et al*. (1989) radioactively labelled ivermectin with tritium. It was found that 40 to 45% of the radioactivity in the dung was from the parent compound, with the rest being metabolites of ivermectin. These metabolites retain their insecticidal activity, although they are less toxic than the parent compound. Therefore, in the dung the pesticide is still active against dung breeding pests such as horn fly, bush fly and face fly (Meyer *et al*., 1980; Schmidt, 1983; Sommer *et al*., 1992).

Elimination of the compound from cattle varies with route of administration. Sommer *et al.* (1992) collected faeces from cattle treated with 200 µg/kg injection or a 500 µg/kg pour-on of ivermectin for up to 43 days after treatment and analysed faecal residues using gas chromatography. The dose peaked after 1 day at 9 ppm dry weight (dw) via the topical route, or around 3 days at 4 ppm dw, if administered subcutaneously (Fig. 1.3). For both treatments residues were undetectable after 13 - 14 days, although the level of detection in this experiment was 0.05 ppm, which is still toxic to insects. In a study by Iwasa *et al.* (2007), ivermectin was detected for 28 to 35 days after treatment with 500 µg/kg pour-on ivermectin when a lower

detection limit of 0.002 ppm wet weight (ww) was achieved. Herd *et al*. (1996) investigated a bolus delivery system, and found ivermectin remained at around 0.5 ppm (ww) in dung throughout the period the bolus was active.

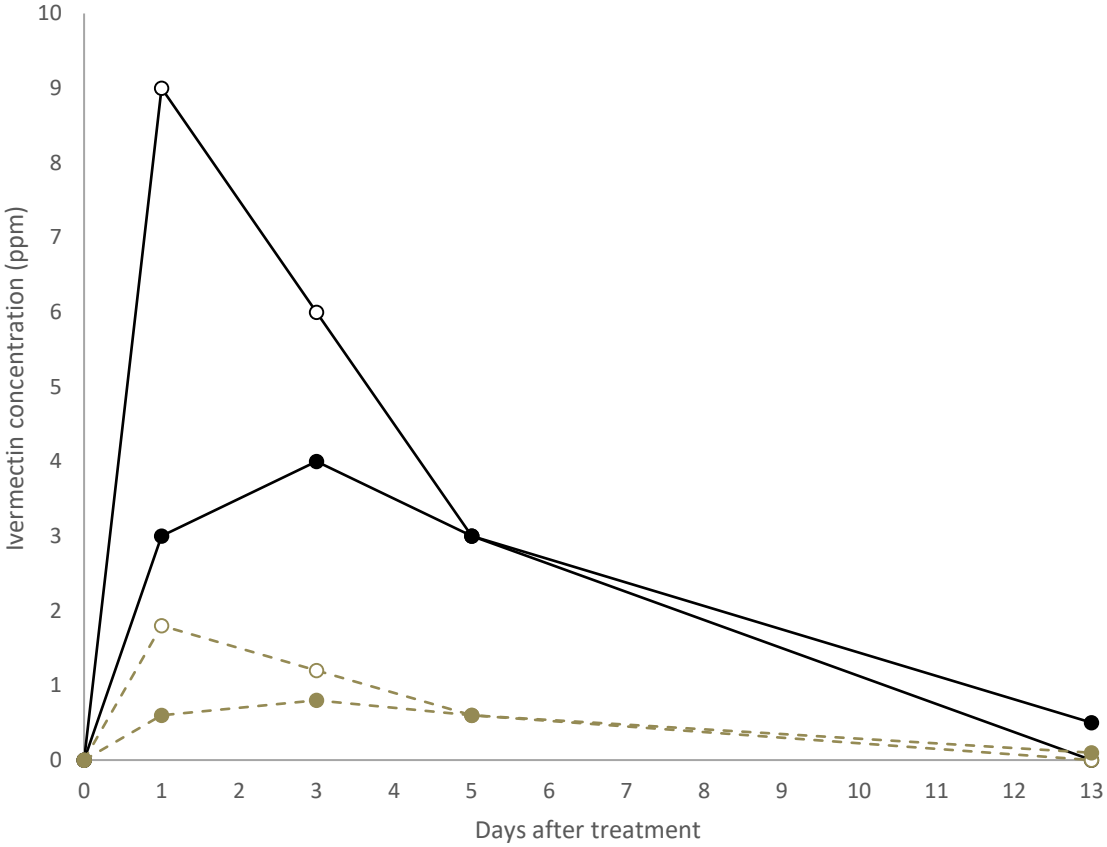

Figure 1.3 Elimination of ivermectin from cattle. Concentrations of ivermectin (ppm) found in dung after treatment with either 500 µm/kg pour-on formula (○) or 200 µm/kg injection (●). Solid lines (—) indicate concentrations from dry weights, dashed lines (- - -) indicate the wet weight concentration, corresponding to dung of 20% dry matter. Adapted from: Sommer *et al.* (1992).

Lumaret *et al*. (2007) treated cattle with a 500 µg/kg pour-on formulation, and detected concentrations which peaked 3 days after treatment at 0.2 – 0.05 ppm of ivermectin. The lower concentrations are due to the fact that concentration was

measured by wet weight of dung. Dung is from 60 to 90% water content of fresh mass, so concentration can be expected to be 6 to 9 times larger when converting wet to dry concentration (Fig. 1.3).

The half-life of ivermectin is relatively long. In the laboratory a half-life in dung of 90 to 240 days has been recorded (Halley *et al.,* 1989). However, ivermectin degrades in sunlight, so its half-life is shorter when experiments are undertaken in the field. A half-life of 1 to 6 weeks has been demonstrated for ivermectin exposed to direct sunlight, depending on conditions and substrate in which the compound is mixed (Halley *et al*., 1989; Sommer *et al.,* 1992).

1.3.2 Synthetic pyrethroids

Pyrethroids are responsible for 18% of the financial value of the overall pesticide market (Pickett, 2004). They are used on crops, animals, and in households and gardens (Saillenfait *et al*., 2015). Synthetic pyrethroids are derived from natural pyrethrethrins, of which pyrethrum occurs naturally in *Chrysanthemum cinerafolis* flowers, although some of the synthetic compounds are only distantly related to the parent compound (Vijverberg & van den Bercken, 1990). Synthetic pyrethroids can be divided in to type I and type II pyrethroids. Type I pyrethroids are analogues of chrysanthemic acid and do not contain a cyano group. Type II pyrethriods do contain a cyano group and are analogues of pyrethric acid. Presence or absence of the cyano group effects the toxological properties of the compound. Popular synthetic pyrethroids include cypermethrin, deltamethrin, cyhalothrin and flumethrin, all type II pyrethroids. They are active against flies, ticks, lice and mites (Floate *et al*., 2005).

Synthetic pyrethroids have been designed to be more potent and have longer half-lives than their parent compounds. They act by inducing a strong excitatory action in the peripheral and central nervous system of insects (Vijverberg & van den Bercken, 1990). Sodium channels are modified forcing them to remain open. This causes hyperactivity, followed by paralysis and possibly death. The effects of type I pyrethroids can be reversed, whereas the depolarisation by type II compounds is irreversible. This is due to the longer duration of modified sodium currents in type II synthetic pyrethroids (Bloomquist, 1996).

In mammals, synthetic pyrethroids also work by binding to voltage-gated sodium channels. Pyrethroids are more toxic to insects due to the reduced sensitivity of mammalian sodium channels to modification by pyrethroids (Vais *et al*., 2001). However, toxicity still occurs in mammals at high concentrations. Acute exposure to type I pyrethroids causes animals to exhibit tremors, whereas type II cause choreoathetosis (irregular contractions and twisting) and salivation (Saillenfait *et al.*, 2015). This, however, is not a comprehensive classification, as some type II compounds will cause type I symptoms.

In cattle, the route of elimination for synthetic pyrethroids is mainly via the faeces; about 95% of the compound is eliminated this way (Venant *et al*., 1990; Floate *et al*., 2005). The proportion of the compound eliminated in the faeces depends largely on the compound applied and route of administration. Products such as deltamethrin and cypermethrin are metabolised more fully than products such as ivermectin. For example, Akhtar *et al*. (1986) found quantities in the dung as low as 36% when deltamethrin was administered orally. However, it is likely that metabolites of synthetic pyrethroids are just as toxic to insects as the parent product.

After administration, a pyrethroid would usually be eliminated in the faeces over around two weeks. For deltamethrin, applied by spray or spot on formulation, wet weight concentration peaks at around 0.4 ppm (Vale *et al*., 2004). Wardhaugh *et al*. (1998) also found a pour-on formulation of deltamethrin peaked in the faeces at 0.4 ppm (ww), three days after treatment. It is likely that synthetic pyrethroids follow similar patterns of elimination to macrocyclic lactones with a higher peak concentration for pour-on formulations than injectable, although this has yet to be confirmed.

The half-life of synthetic pyrethroids depends on their exposure to sunlight, heat and moisture. Half-lives can vary from a few days to months. A half-life of 2 to 12 weeks has been demonstrated, depending on the compound used and the soil type (Miyamoto, 1981). Vale *et al.* (2004) found when dung was spiked with 10 ppm (ww) that there was no change in the dung concentration over a 2-month period of field exposure in Zambia. However, this concentration is far higher than would be found if compounds are applied in compliance with the manufacturer's instructions.

1.4 - Lethal and sublethal effects to dung fauna

When pesticides first began to be widely used they were viewed as the solution to many of mankind's problems; reducing the prevalence of disease and increasing crop and livestock yields. However, in 1962 Rachel Carson published Silent Spring, a book highlighting the indiscriminate effects of pesticides. This book brought the attention of the general public to the damage pesticides were causing to non-target species. The book began a movement which led to many substances being banned for agricultural use and stricter legislation when designing new pesticides. For example, DDT, a once widely used compound, became linked with the thinning of raptor egg shells in the U.K., amongst other negative effects on wildlife (Ratcliffe, 1967). The pesticide became banned in many nations, including the U.S. and U.K.

Despite stricter legislation over the compounds used for pest and parasite control, many that are currently widely employed have negative impacts on non-target organisms. For example, neonicotinoids are the most widely used pesticides in the world, yet there is good evidence of lethal and sublethal effects on bees at environmentally relevant concentrations (Goulson, 2013). The high concentrations of macrocyclic lactones and synthetic pyrethroids found in dung have been reported to be active against beneficial dung-dwelling insects (Madsen *et al*., 1990; Fincher, 1992; Sommer *et al*., 1992; Wardhaugh *et al*., 2001). The effect of macrocyclic lactones and synthetic pyrethroids on dung fauna has a knock-on effect on the services that they provide in pasture systems. Effects can be lethal or sublethal, depending largely on life stage.

1.4.1 Macrocyclic lactones

***Lethal effects*:**

The abundance and diversity of dung fauna in dung pats is affected when cattle are treated with macrocyclic lactones. Wall and Strong (1987) were the first to study the effects of ivermectin on dung dwelling insects. A bolus treatment, releasing ivermectin at 40 µg/kg per day, eliminated almost all Coleopteran and Dipteran fauna, preventing decomposition. A similar study by Lumaret *et al.* (1993) found significantly fewer beetles were present in dung from cattle treated with a subcutaneous injection of ivermectin at 200 µg/kg. The number of beetles

increased with the number of days post-injection. Other studies have illustrated the same effect in different climates, and using varying methods of application (Sommer *et al*., 1992; Basto-Estrella *et al*., 2014; Tonelli *et al.*, 2017). Bioassays, conducted in the laboratory and the field, confirm the lethal effects of macrocyclic lactones on dung beetles, as described below.

Adult mortality:

For experiments, dung is usually either collected at intervals from cattle treated with a macrocyclic lactone or it is spiked with the compound at various concentrations. Standard treatments of macrocyclic lactones are 200 µg/kg injection or 500 µg/kg of a pour-on formulation. Therefore, where dung is collected from treated animals, residues in dung can be expected to be similar to those as displayed in Fig. 1.3, peaking at 2 ppm (ww), after a treatment to cattle of pour-on formulation.

The vast majority of studies focus on ivermectin, as it is one of the most common anthelmintics used for pest and parasite control. Perez-Cogollo *et al*. (2015) found 80% adult mortality of *Onthophagus landolti* (Harold) when exposed to 1 ppm (ww) of ivermectin and 30% mortality when beetles were exposed to 10 ppm (ww) after 10 days. Similar results were found with a tropical beetle species, *E. intermedius*. Laboratory studies with spiked dung observed 50% mortality at 1 ppm and 100% mortality at 100 ppm, after 10 days (Cruz Rosales *et al*., 2012). However, a concentration of 100 ppm is not environmentally relevant if the compound is applied according to manufacturer's instructions.

In contrast, other studies have found no effect of ivermectin on mortality of adult beetles. For example, no effect was found of ivermectin on survival of Mediterranean paracoprids: *Onthophagus taurus* (Schreber) and *Euoniticellus fulvus* (Goeze). This was found using dung collected at intervals after treatment with a control release capsule, although the dose of the capsule per day was unspecified (Wardhaugh *et al*., 2001). The same was found for *E. fulvus*, using dung collected after cattle had received a 500 µg/kg pour-on treatment of ivermectin (Wardhaugh *et al.*, 1993). Wardhaugh and Rodriguez-Menendez (1988) found adult *Bubas bubalus* (Olivier) and *Copris hispanus* (L.) suffered no significant mortality when exposed to dung from cattle which had been administered with a 200 µg/kg

injection of ivermectin. In a field trial Lumaret *et al.* (1993) also found no effect of a 200 µg/kg injection of ivermectin to cattle on mortality of adult dung beetles.

Ridsdill-Smith (1988) similarly found no effect of avermectin on mortality of *Onthophagus binodis* (Thunberg) in Australia. However, this study was poorly designed; dung was collected 1, 2, 4, 8 and 11 weeks after cattle were treated with a 200 µg/kg injection of avermectin B1. These collection times miss the period when residue concentrations in dung would be at their highest, making the study of limited value. Additionally, no control was used. Nevertheless, immature beetles were also tested, and significant mortality was observed when fed dung collected 1 week after treatment. Other studies support the idea that immature beetles may be more susceptible than mature adult beetles to MLs. For example, no mortality of *C. hispanus* was observed in mature adult beetles, but newly emerged beetles suffered 90% mortality when fed dung collected 3 days after the administration of cattle with 200 µg/kg ivermectin injection (Wardhaugh & Rodriguez-Menendez, 1988). Comparable results have been found for *E. fulvus* (Wardhaugh *et al.*, 1993).

Other studies have focused on alternative macrocyclic lactones, finding that mortality rates vary depending on the compound administered. For example, Dadour *et al*. (2000) studied newly emerged *O. binodis* and found a mortality rate of up to 35% using dung from cattle given a 200 µg/kg injection of abamectin. Doramectin only had an effect using dung collected up to 9 days after the 200 µg/kg injection, causing a mortality rate of 20%. Lastly, a 500 µg/kg pour-on treatment of eprinomectin negatively impacted the survival of newly emerged *O. taurus* beetles, but moxidectin, of 500 µg/kg pour-on treatment, did not have an effect (Wardhaugh et al., 2001).

Larval mortality:

While studies with adult beetles report variable effects, most demonstrate that immature beetles are more highly susceptible to ML residues in dung than adult beetles. Differences are also seen between the adult and larval stage in susceptibility. This difference can likely be attributed to differences in feeding habits. Adult beetles filter out larger particles, mainly consuming the nutrient-rich liquid matter of dung (Hanski & Cambefort, 1991). In contrast, dung beetle larvae feed on larger particles of dung, using biting mouthparts. MLs tend to bind to large

soil particles so larvae are likely to be exposed to higher doses (Steel & Wardhaugh, 2002).

When testing paracoprid species, studies have shown that ivermectin can still cause larval mortalities using dung up to 32 days post-injection of 200 µg/kg (Wardhaugh & Rodriguez-Menendez, 1988). Mortality of larval *O. binodis* was 100% using dung from 6 days post injection with 200 µg/kg of avermectin B1 (Ridsdill-Smith, 1988). *E. intermedius* larvae show high levels of mortality when exposed to ivermectin; at 1 ppm 60% of larvae died and those that survived only attained the first instar (Cruz Rosales *et al*., 2012).

With endocoprid beetles, *Aphodius* larvae were reduced in number in dung collected up to 2 days after treatment with ivermectin by both 200 µg/kg injection and 500 µg/kg pour-on formulation (Sommer *et al.*, 1992). Strong and Wall (1994) found higher *Aphodius* larval mortality than the control when exposed to dung of cattle for up to 7 days after treatment with 200 µg/kg ivermectin, but not when moxidectin was used at the same dose.

Sublethal effects:

Sublethal effects include reduced emergence, increased development time and reduced feeding rates. Sublethal effects may have important long-term effects on dung beetle populations.

Emergence:

Kruger and Scholz (1997) looked at the emergence of adults of two tropical species, *E. intermedius* and *O. alexis*. They exposed larvae to dung from cattle treated with ivermectin injection at 200 µg/kg. Emergence of *O. alexis* was reduced when larvae were exposed to dung collected for up to 7 days after treatment, by around 75%. In contrast, emergence of *E. intermedius* was completely prevented when exposed to dung collected for up to 7 days after treatment. Using dung 21 days after treatment, emergence was still reduced by 30% when compared to the control. A study by Fincher (1992) found no emergence of *O. gazella* from brood balls made from dung 1-week post treatment of cattle with 200 µg/kg of ivermectin.

Other macrocyclic lactone compounds have also been tested. Dadour *et al*. (2000) noted that adult *O. binodis* only emerged from brood masses made from dung

collected 42 days after a standard injection of 200 µg/kg abamectin to cattle. Emergence was prevented for 3 to 6 days when 200 µg/kg of doramectin was used. A 200 µg/kg injection of moxidectin had no effect on emergence of *E. intermedius* and *O. gazelle* (Fincher & Wang, 1992).

Endocoprid species again seem more resistant; no significant difference was found in emergence of *A. haemorrhoidalis* when cattle were treated with 200 µg/kg injection of ivermectin and moxidectin in cattle dung (Kadiri *et al*., 1999). However, the emergence of *Aphodius constans* (Duftschmid) was reduced for two days when sheep dung was used.

Development:

Studies have reported delays in the development of dung beetles in the presence of macrocyclic lactones. Kruger and Scholz (1997) reported that development times of *E. intermedius* and *O. alexis* were prolonged after they were exposed to dung from cattle treated with 200 µg/kg of ivermectin, collected for up to 28 and 21 days after treatment, respectively. Development time for *E. intermedius* was 2.5 times longer in dung collected 1 to 14 days after treatment. Cruz Rosales *et al.* (2012) found a similar effect on *E. intermedius;* when larvae were exposed to dung that had been spiked with 0.01 ppm ivermectin a delay of 0.5 times was found. A delay in development has also been reported for *E. fulvus* (Lumaret *et al.,* 1993).

Madsen *et al*. (1990) found that development was inhibited for *Aphodius* larvae exposed to dung collected for up to 1 day after treatment when cattle were injected with a 200 µg/kg dose of ivermectin. However, dung collection dates of 1, 10, 20 and 30 days after treatment were used, so most of the dung collection occurred after the period when peak ivermectin concentrations in the dung would have been anticipated (Fig. 1.3). Strong and Wall (1994) also found a 200 µg/kg ivermectin injection to cattle inhibited development of *Aphodius*, when exposed to dung collected for up to 7 days after treatment. In contrast, there was no difference between the control and treatment group when moxidectin was used.

Reproduction:

Kruger and Scholz (1997) found a reduction in the fecundity of *E. intermedius* in the first breeding week using dung collected from 1 to 14 days after an injection of 200 µg/kg ivermectin to cattle. The fecundity of *E. intermedius* was 2.8 times greater in

the control group, when dung was used 14 days post-treatment. Cruz Rosales *et al.* (2012) also found significantly fewer brood masses oviposited by *E. intermedius* when dung was spiked with ivermectin. Dung spiked at 1 ppm (ww) of ivermectin reduced brood ball production by 4.5 times and reduced the mean weight of brood masses. Reduced brood ball production was also found in *C. jessoensis* in the laboratory when fed dung from cattle which had been treated 7 days previously with 500 µg/kg ivermectin pour-on formulation, although there was no difference in the weight of the brood balls (Iwasa *et al*., 2007). Martinez *et al*. (2017) looked at reproduction in *E. intermedius* in response to ivermectin spiked in to dung from 0.003 to 0.3 ppm (ww). They found a significant relationship between concentration of ivermectin and basal oocyte size; at high concentrations of ivermectin the basal oocyte became smaller in size. Additionally, the number of oocytes along the coiled germarium increased. In contrast, Fincher (1992) found no significant difference in brood ball production from *E. intermedius* and *O. gazelle* after cattle were injected with 200 µg/kg ivermectin, although emergence from the brood balls was reduced.

Other studies used alternative macrocyclic lactones. Doramectin and abamectin both were found to reduce both number of oocytes and length of the basal oocytes significantly in *O. binodis* (Dadour *et al*., 2000). For doramectin, this was after exposure of beetles to dung collected between 2 to 6 days after treatment of cattle with a 200 µg/kg injection. For abamectin, oocytes were reduced after adults were exposed to dung collected 42 days after a 200 µg/kg injection. Brood ball production was affected in a comparable way. Fincher and Wang (1992) tested moxidectin on *E. intermedius* and *O. gazelle*, finding no significant differences in brood ball production. Iwasa *et al.* (2008) also noted no significant difference in brood ball production or weight for *C. jessoensis* when moxidectin was used. Wardhaugh *et al*., (2001) found brood production was suppressed by eprinomectin in *O. taurus*, when cattle were given a dose of 500 µg/kg pour-on formulation.

Other:

A reduction in feeding activity has also been reported. Finnegan *et al*. (1997) reported that dung beetles consume sporangia as they feed. Therefore, to measure feeding activity they measured reduction in sporangia when *Aphodius sphacelatus* (Panzer) dung beetles were exposed to dung spiked with 1 ppm ivermectin (ww).

When exposed to ivermectin fewer sporangia were consumed. Other research supports this as dung beetles fed spiked dung have empty guts and reduced fat bodies (Martinez *et al.,* 2017).

Transgenerational effects have also been reported. Adults exposed to low concentrations of ivermectin (0.01 ppm ww), and then moved to clean dung to breed, were found to have a 50% lower egg hatch and, for those larvae that pupated and emerged as adults, male horns were significantly shorter (Baena-Díaz *et al.*, 2018).

1.4.2 Synthetic pyrethroids

The effects of synthetic pyrethroids on dung fauna are less extensively studied than those of the macrocyclic lactones, despite their widespread use.

Adult mortality:

Several studies in the field have found that residues of synthetic pyrethroid in dung cause significant mortality of adult dung beetles compared to the control treatment. Sands *et al.* (2018) found this for deltamethrin in Botswana, as did Chihiya *et al.* (2006) in England and Vale *et al*. (2004) in Zimbabwe. Dead dung beetles were found in and surrounding the dung pats containing SP residues, suggesting toxicity.

In the laboratory a similar pattern is seen. Sands *et al.* (2018) found that for the paracoprid beetle, *Metacatharsius* troglodytes (Boheman), the time taken for 50% of the beetles to die decreased at high concentrations of deltamethrin spiked into dung. At the highest concentration of 1 ppm it took 6 days for 50% of the beetles to die. These results were confirmed using dung collected from treated animals. Significantly more mortalities occurred, compared to the control group, after exposure of *O. binodis, O. alexis* and *E. fulvus* to dung collected for up to 6 days after treatment of cattle with 750 µg/kg deltamethrin pour-on (Wardhaugh *et al*., 1998). Vale *et al.* (2004) compared pour-on and dip formulations of deltamethrin spiked into dung on a range of dung beetle species, including *Copris*, *Digitonthophagus*, *Onitis* and *Sisyphus* spp. This study found that the LC_{50}, lethal concentration, where there is 50% mortality of dung beetles, was 0.04 ppm (ww) for deltamethrin pour-on and 0.25 ppm (ww) for deltamethrin dip. Other studies

have also reported pour-on treatments to be more toxic than sprays (Bianchin *et al.*, 1997).

Experiments with other pyrethroids in dung have demonstrated significant effects on mortality. Mortality of *E. intermedius* was recorded in the presence of cypermethrin or flumethrin pour-on at 1000 µg/kg (Kruger *et al*., 1999). In the presence of cypermethrin, mortality was up to 100% when using dung collected from cattle between 2 to 7 days after treatment. Flumethrin did not significantly affect mortality. Another study also found a significant effect on the mortality of adult *Copris tripartitus* (Waterhouse) in the presence of cypermethrin, but only in dung collected 1 day after spray-on treatment of cattle of 2.1 g per steer (Bang *et al.*, 2007). In contrast, a study by Kruger *et al.* (2006) found no significant effect on adult beetle mortality in dung collected from cows that had been treated with cypermethrin pour-on at 1000 µg/kg. Bianchin *et al.* (1998) found a significant effect on the mortality of *O. gazelle* in the presence of alphamethrin, cyalothrin, deltamethrin and flumethrin pour-on treatment at 1000 µg/kg in the dung of treated cattle; in all cases there was a significant increase in mortality using dung collected up to 8 days after treatment. For dung collected more than 8 days after treatment, there was no significant difference in beetle mortality between the treatment groups and the control.

Larval mortality:

Dung beetle larvae were only present in dung collected more than 7 days after treatment of cattle with a pour-on formulation of deltamethrin (Mann *et al*., 2015). The study did not specify the dose, only that the manufacturer's instructions were followed. In a mesocosm experiment approximately 50% fewer larvae were found in dung pats containing 0.1 ppm (ww) deltamethrin (Sands *et al*., 2018). In contrast, larval survival was reported not to be affected in *E. intermedius* when exposed to dung from cattle treated with cypermethrin or flumethrin pour-on at 1000 µg/kg (Kruger *et al.,* 1999). Larval beetle survival was also not affected when exposed to dung collected from cattle treated with spray-on cypermethrin when each steer was treated with 2.1 g (Kryger *et al*., 2006).

Sublethal effects:

In contrast to lethal effects, sublethal effects can occur weeks, rather than days after treatment.

Development time and reproduction:

When dung was spiked with 0.1 ppm (ww) of deltamethrin 40% fewer brood balls were produced, at 1 ppm (ww) brood ball production was reduced to 20% that of the control (Sands *et al.*, 2018). Additionally, no unhatched eggs were found in brood balls made from clean dung, but they were found when brood balls were made from spiked dung, although this difference was not significant.

Effects of deltamethrin were tested on *Copris*, *Digitonthophagus*, *Onitis* and *Sisyphus* spp. (Vale *et al*., 2004). Only *Sisyphus* were able to produce brood balls in the presence of deltamethrin, and still no brood balls were produced when beetles were exposed to dung spiked with concentrations of 1 ppm of deltamethrin. Development time increased and fecundity decreased in comparison to the control when *O. binodis* and *E. fulvus* were exposed to deltamethrin residues, when cattle were treated with 750 µg/kg deltamethrin pour-on (Wardhaugh *et al*., 1998). Flumethrin also reduced the number of brood balls produced by *E. intermedius* when using dung collected 7 days after the treatment of cattle with 1000 µg/kg (Kruger *et al*., 1999). Lastly, cypermethrin prevented brood ball production when beetles were exposed to dung collected 1 day after cattle treatment with 2.1 g per steer of spray-on treatment and, reproduction was reduced when beetles were provided with dung collected 3 and 5 days after cattle treatment (Bang *et al*., 2007). Additionally, development of the basal oocyte was suppressed. However, when the testicular follicle was measured there was no significant difference between treatments.

1.5 Aim

The overall aim of the work described in this thesis, was to gain an understanding of how macrocyclic lactones and synthetic pyrethroids affect the reproductive physiology and fecundity of temperate dung beetle species. Specifically, the study asked two questions: first, does the experimental addition of

sublethal doses of ivermectin to dung affect the reproductive output and physiology of dung-dwelling beetles and second, does long-term use of macrocyclic lactones and synthetic pyrethroids in beef herds have an impact on dung beetle reproductive development and output.

Chapter 2 – Effects of sublethal doses of ivermectin on the reproductive output and physiology of two temperate dung beetle species

2.1 Introduction

Dung-colonising beetles play an important role in pasture systems by aerating cattle dung and removing it to below ground (Beynon *et al*., 2015). However, endectocides applied to cattle, to control pests and parasites, pass out into dung in concentrations which are toxic to insects (Sommer *et al*., 1992). While the toxic effects of endectocides on dung beetles have been well studied, the fact that dung beetles may suffer sublethal effects in response to endectocide residue exposure has rarely been investigated (Wardhaugh & Rodriguez-Menendez, 1988; Kruger & Scholz, 1997; Perez-Cogollo *et al*., 2015). In consequence, the ecosystem services dung beetles provide may be more severely compromised than indicated simply by an understanding of lethal effects (Manning *et al*., 2017; Verdu *et al*., 2018).

The macrocyclic lactone, ivermectin is the focus of many studies as it is the most widely used avermectin (Campbell *et al*., 1989). Its effects on beetle reproductive physiology and output are of particular importance, as they determine the number of individuals in the next generation and therefore, population abundance. Studies on reproductive sublethal effects have focused primarily on tropical paracoprid species (Cruz Rosales *et al.*, 2011; Martinez *et al*., 2017). The response of the reproductive physiology of temperate paracoprid and endocoprid species to ivermectin are poorly understood. Endocoprid species have different oviposition behaviour to paracoprid species, spending their whole lifecycle breeding and feeding in dung and/or the dung-soil surface (Fig. 1.1). In contrast, paracoprid beetles bury brood balls of dung in which they lay eggs. Their different oviposition behaviours could allow differential exposure to ivermectin residues and therefore result in differing susceptibility. Additionally, paracoprid *Onthophagus* and endocoprid *Aphodius* genera have different ovary physiology, which, again, could result in different susceptibility.

This study aimed to address the question: does the experimental addition of sublethal doses of ivermectin to dung affect the reproductive output and physiology of two dung beetle species: *A. prodromus* and *O. similis.* These are two common northern temperate species of dung-colonising beetle, the former an endocoprid and the latter a paracoprid.

2.2 Methods

2.2.1 Traps

Trapping took place from 9th – 13th May 2018 at Failand farm, Bristol, a registered organic farm. Twenty pitfall traps were set up in total, in a field adjacent to grazing cattle (Fig. 2.6). Traps were positioned approximately 10 meters apart. All traps were set up between 9am and 11am.

Pitfall traps comprised a bucket (18 cm depth x 16 cm diameter), buried to rim level in soil (Fig. 2.4; Fig. 2.5). The soil was packed tightly to the bucket rim to ensure capture of walking dung beetles. The dung bait was placed on chicken wire mesh, folded over 3 times over the top of the bucket (approx. 30 x 30 cm). A 20 cm diameter flower pot was used to form a 1 kg dung pat for each trap. The bait covered the opening of the bucket to prevent the exit of beetles. Traps were protected from rain by 15 cm diameter aluminium pie dishes, supported using 30 cm bamboo canes, dug in to the soil.

Dung for baiting the traps, and for use in the experiment, was also obtained from Failand farm. Dung was collected from a field containing beef cattle, adjacent to the field used for trapping. Only fresh dung, without a crust, was collected to minimize previous insect colonisation. All dung was from Red Poll cattle which had recently been turned out on to pasture. They had not been treated with any endectocides that year. After collection, an industrial paddle mixer (Silverline 850W) was used to homogenise the dung for 15 min. Dung was stored at 4 °C before use. Dry weight analysis of a subsample, using sequential drying in an oven followed by reweighing, showed that the dung for use in the experiment was 11.5% dry mass. For pitfall trapping, chopped up hay was added to the dung to thicken the consistency to allow it to sit on chicken wire. Bait was replaced with fresh dung

every other day. On days where dung was not replaced, the crust was peeled off and discarded.

Beetles were collected from traps daily and placed into terraria, made from food-grade plastic containers (8 cm, 11.5 cm, 17 cm). These contained 400 g of clean, nontoxic children's play sand (Argos Ltd) and 250 g of dung. The sand was kept dry as the dung contained sufficient moisture. Holes were punched in the lid to allow for air exchange. The containers were stored at 4 °C. At this temperature beetles were relatively inactive.

The two most abundant species recorded in the pitfall catches were selected for the experiment: *O. similis* and *A. prodromus*. Beetles were identified using identification guides from Jessop (1986) and Skidmore (1991). Other beetles were discarded. Before the experiment commenced, beetles were starved for 24 h.

2.2.2 Bioassay

Where possible, methodology followed the OECD guidance on dung-beetle toxicity testing (OECD, 2010). Experimental terraria, as described above, were maintained at 20 °C, on a 12:12 light:dark cycle in an illuminated cooled incubator (Sanyo Electric, Osaka City, 540-6226, Japan). Each terrarium was supplied with 250 g of dung spiked with ivermectin at concentrations of 0.01 ppm, 0.1 ppm and 1 ppm in wet dung. *Aphodius prodromus* had additional terraria with dung spiked at the concentration of 10 ppm, as the species was in greater abundance in the pitfall traps. In terms of dry weight, the concentrations equated to 0.087 ppm, 0.87 ppm, 8.7 ppm and 87 ppm. The dung for the control group was spiked with ethanol only. *Aphodius prodromus* also had a deionised water control, to control for the possible effect of ethanol, due to its greater abundance. Ivomec Super® (Boehringer Ingelheim Limited, Berkshire) was used to spike the dung, which comprised 1% w/v ivermectin. Ivomec Super® also contains 10% w/v clorsulon; this is a narrow-spectrum flukicide so is inactive against insects. Dilutions were made by mixing ivermectin in 10 ml of ethanol solvent per kg of dung. In the deionised water control 10 ml of water was used per kg of dung. The solution was mixed into the dung thoroughly for 10 minutes using a spoon and glass rod to ensure the substance was thoroughly homogenised throughout the dung. To evaporate off the solvent, dung was left for 1 h before use.

For *O. similis,* each terrarium was populated with 13 beetles of approximately equal sex ratio. Dominant males (those with a horn) were also divided evenly between the groups. Once divided, beetles were randomly allocated to treatments. For *A. prodromus*, sex could not be determined without dissection, so individuals were allocated in to groups of 25 individuals at random. For both species, each treatment was repeated 3 times. The experiment continued for 3 weeks in total, with dung replaced weekly. At the end of every week, dead dung beetles were counted, removed and stored in ethanol. After three weeks, all remaining beetles were killed and stored in ethanol.

2.2.3 Dissections

In total 221 females were dissected. Dissections were carried out in Ringer's solution (EMD Millipore Corporation, Billerica MA 01821, USA) under a GX dissecting microscope (GT Vision Ltd, Standsfield, CO10 8LY). Dissections were carried out on all female beetles, irrespective of time of death. Females were designated a reproductive state: gravid or non-gravid. Individuals were defined as gravid if oocytes were differentiated. Individuals were defined as non-gravid if oocytes were undifferentiated, so that in *O. similis* the coiled germarium was one mass or in *A. prodromus* the oocytes had not formed rounded ends. Oocyte counts and measurements were only taken for gravid individuals. For *O. similis*, the basal oocyte was measured; the most mature oocyte at the base of the coiled germarium, nearest to the posterior (Fig. 1.2). A count was also taken of the total number of maturing oocytes. For *A. prodromus*, a mature oocyte was picked at random for measurement. The number of oocytes was also counted. Additionally, the left hind leg was removed for measurement of the tibia. This measurement was used as an indication of beetle size; it was assumed that tibia length and body size varied isometrically. The hind tibia of all females was measured, regardless of reproductive state.

Pictures of the hindleg and ovaries were taken using a Lecia MZ12 dissecting microscope and Leica DFC295 camera (Leica Microsystems Ltd, Milton Keynes, MK14 6FG). Any measurements were taken using the Leica Application Suite V3.8, with the measurement tool.

2.2.4 Data analysis

Data were analysed using the statistical package, RStudio (Version 3.4.4). Binary logistic regression was used to analyse whether treatment had an impact on female reproductive condition (designated as gravid or non-gravid). A one-way ANOVA was used to assess differences in oocyte length and number between treatment groups of gravid individuals. Finally, a test of association Pearson's chi squared test was carried out, to determine whether the mortality rates of individuals in the first week of the experiment differed between the ivermectin concentration treatments.

Figure 2.4 Finished pitfall trap, along with rain guard, used to trap dung-dwelling insects.

Figure 2.5 The construction of a pitfall trap used to catch dung-dwelling insects. From left to right: hole to bury bucket, bucket in hole, chicken wire to hold dung bait, 1 kg pat former, baited trap, evidence of activity in dung.

Figure 2.6 Position of 20 dung-baited pitfall traps on Failand farm, Bristol. Each trap is approximately 10 m apart. The traps are positioned adjacent to a field containing grazing cattle.

2.3 Results

2.3.1 Onthophagus similis

Observations:

Observations indicated that at the lower concentrations of ivermectin the fat body was large and white to light yellow. At higher concentrations of ivermectin, the fat body was smaller and darker yellow. At the highest concentrations, the fat body and oocytes became brown (Fig. 2.8). Additionally, at higher concentrations of ivermectin, beetles appeared to be slower in their movements, taking longer to become active when removed from the dung for counting.

Mortality rate:

During the three-week trial, in the control group 3.7% died. In comparison, no beetles died when exposed to 0.01 ppm of ivermectin, 4.8% died when exposed to 0.1 ppm ivermectin and 7.5% died when exposed to 1 ppm ivermectin (n = 90). A statistical test could not be undertaken on mortality rates of *Onthophagus*, therefore, as too few individuals died for the test to be valid.

Reproductive physiology and fecundity:

In total 90% of the female *Onthophagus* were gravid and each gravid female matured an average of 7.5 (±1.9) oocytes. The binary logistic regression showed that treatment could not predict female reproductive state: gravid or non-gravid ($\chi^2 = 2.63$, $df = 3$, $p = 0.45$), and the number of mature oocytes in gravid females did not differ between the treatment groups ($F_{3,77} = 0.78$, $p = 0.51$). However, there was a significant difference in oocyte length between the treatment groups (Fig. 2.7; $F_{3,77} = 3.66$, $p = 0.016$). Tukey post hoc tests showed that there was no significant difference between oocyte length of gravid females in the control and the two lowest treatment groups ($p > 0.1$). However, there was a significant difference in oocyte length between the control and the highest treatment group ($p < 0.02$), and between the lowest and the highest treatment group ($p < 0.05$). There was no relationship between size of hind tibia and oocyte length.

2.3.2 Aphodius prodromus

Mortality rate:

The concentration of ivermectin influenced the mortality rates of *Aphodius* in the first week of the experiment (χ^2 = 27.50, *df* = 5, $p < 0.01$; Fig. 2.9). However, there were high rates of mortality in all treatment groups and the water control treatment had particularly high mortality of 50%. By the end of the 3-week experiment, only 2% of individuals remained alive. A post hoc test showed there was greater mortality in the water control group than the ethanol control treatment ($p < 0.01$), and the treatments of 0.01 ($p < 0.01$), 0.1 ($p < 0.001$) and 10 ppm ($p = 0.01$). There was also a significant difference between the treatments of 0.1 and 1 ppm; greater mortality occurred at the higher concentration of ivermectin ($p = 0.03$).

Reproductive physiology and fecundity:

The mean number of oocytes per gravid female *A. prodromus* was 7.0 (±4.2). The binary logistic regression showed that treatment was a significant predictor of female reproductive state (χ^2 = 14.80, *df* = 5, $p = 0.011$), with the lowest proportion of gravid females occurring at the higher concentrations of ivermectin (Fig. 2.10). However, only 38% of the ethanol control group was gravid, in comparison to 69% found in the water control group. There was no significant difference in number of oocytes matured by gravid females between treatments ($F_{5, 58} = 0.55$, $p = 0.74$), or difference in oocyte length between treatments ($F_{5, 58} = 0.96$, $p = 0.21$). The mean length of a mature oocyte was 1.21 mm (±0.17). There was no relationship between size of hind tibia and oocyte length.

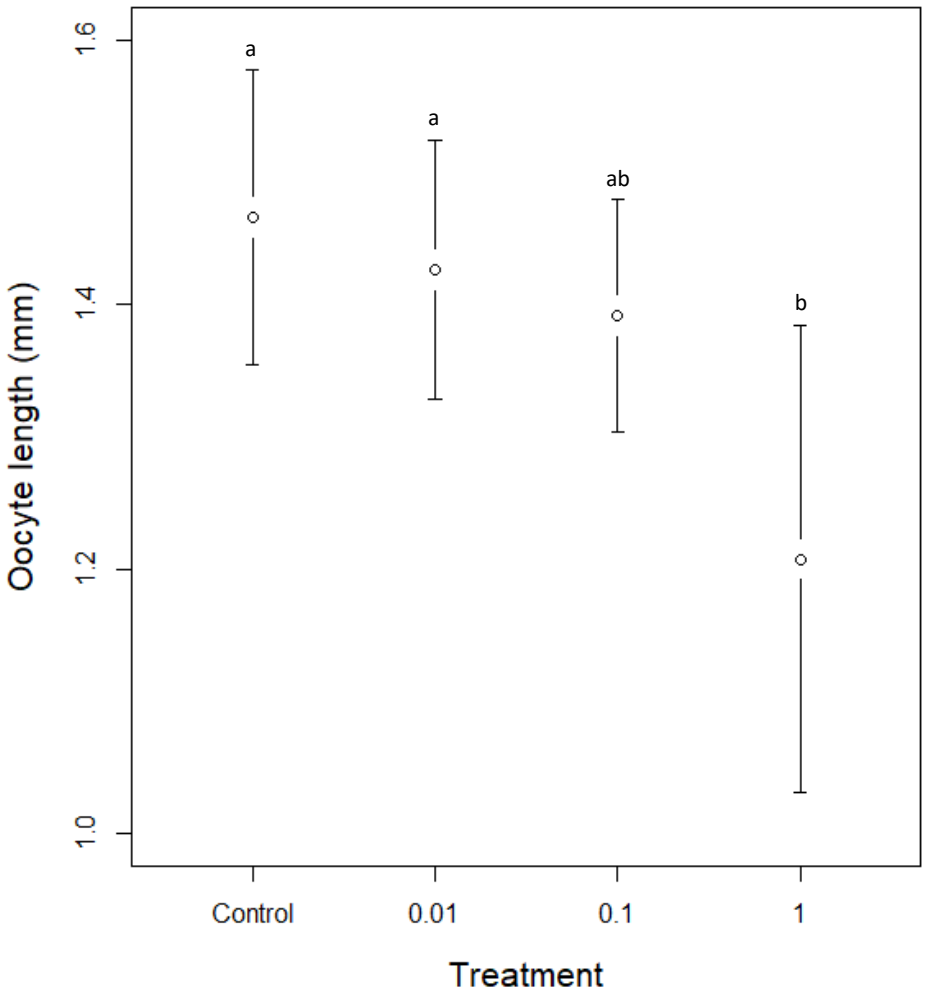

Figure 2.7 The mean oocyte length (±95% confidence intervals) of gravid *Onthophagus similis* after three weeks, when exposed to dung treated with ethanol only (control) or ivermectin at 0.01. 0.1, or 1 ppm. Letters above bars indicate significant differences between means as indicated by Tukey Multiple Range tests.

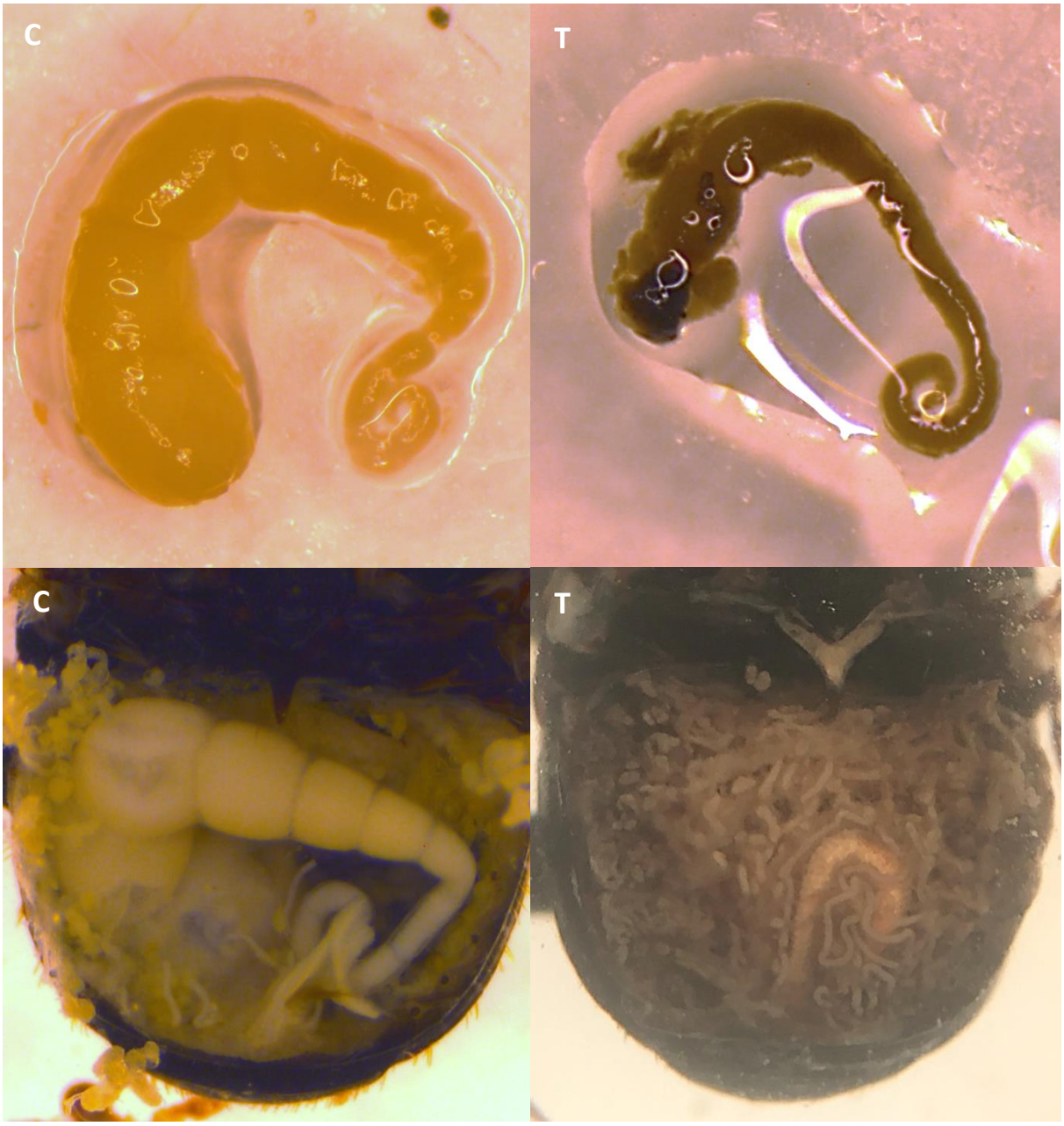

Figure 2.8 Ovary of *Onthophagus similis* when exposed to dung treated with ethanol only (control - C) or ivermectin at 1 ppm (T). Dung beetles were exposed for 3 weeks, with dung replaced weekly.

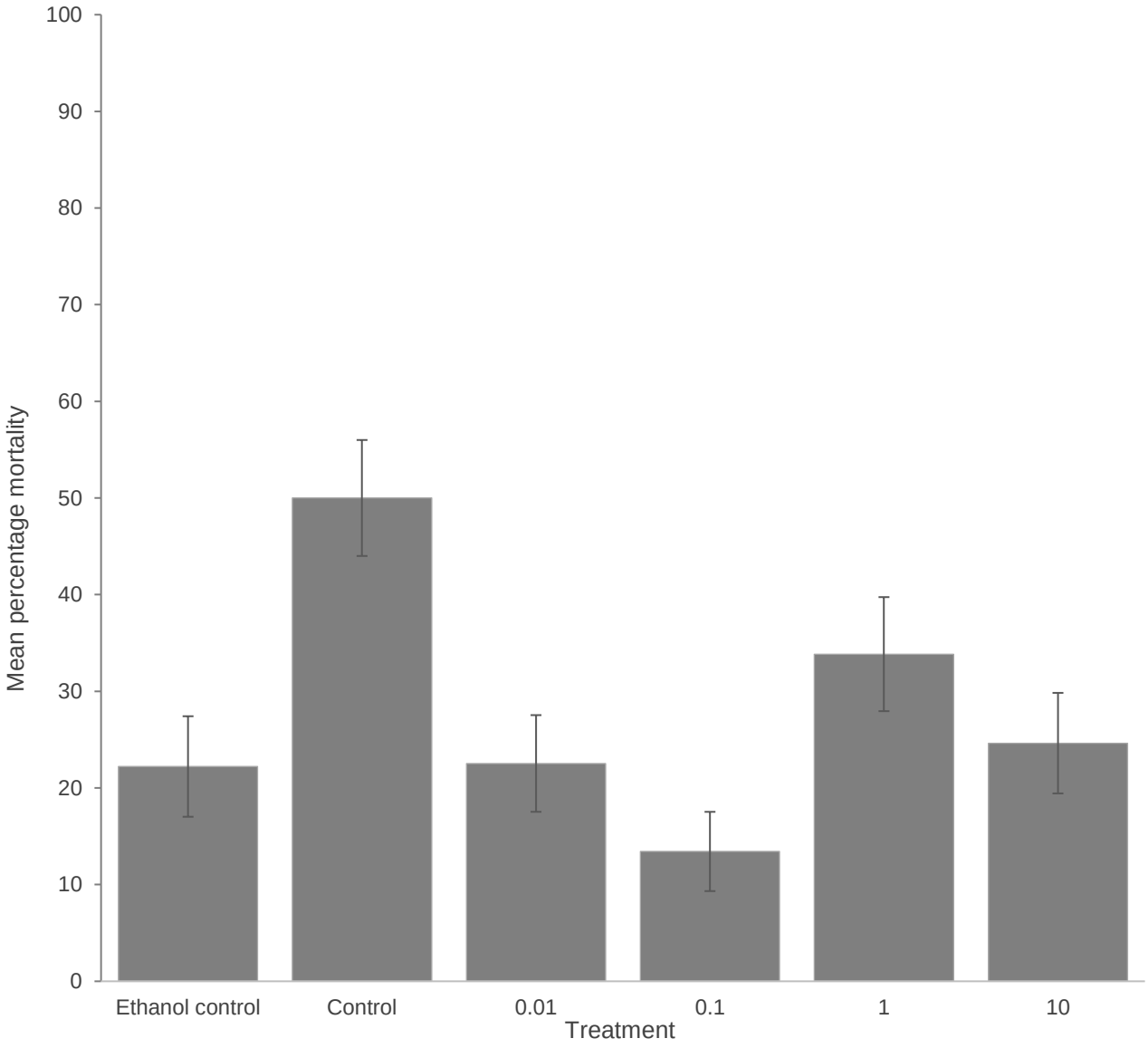

Figure 2.9 The mean (± SE) percentage mortality of *Aphodius prodromus* dung beetles after a period of 1 week when exposed to dung treated with ethanol only, water (control) or ivermectin at 0.01. 0.1, 1 or 10 ppm.

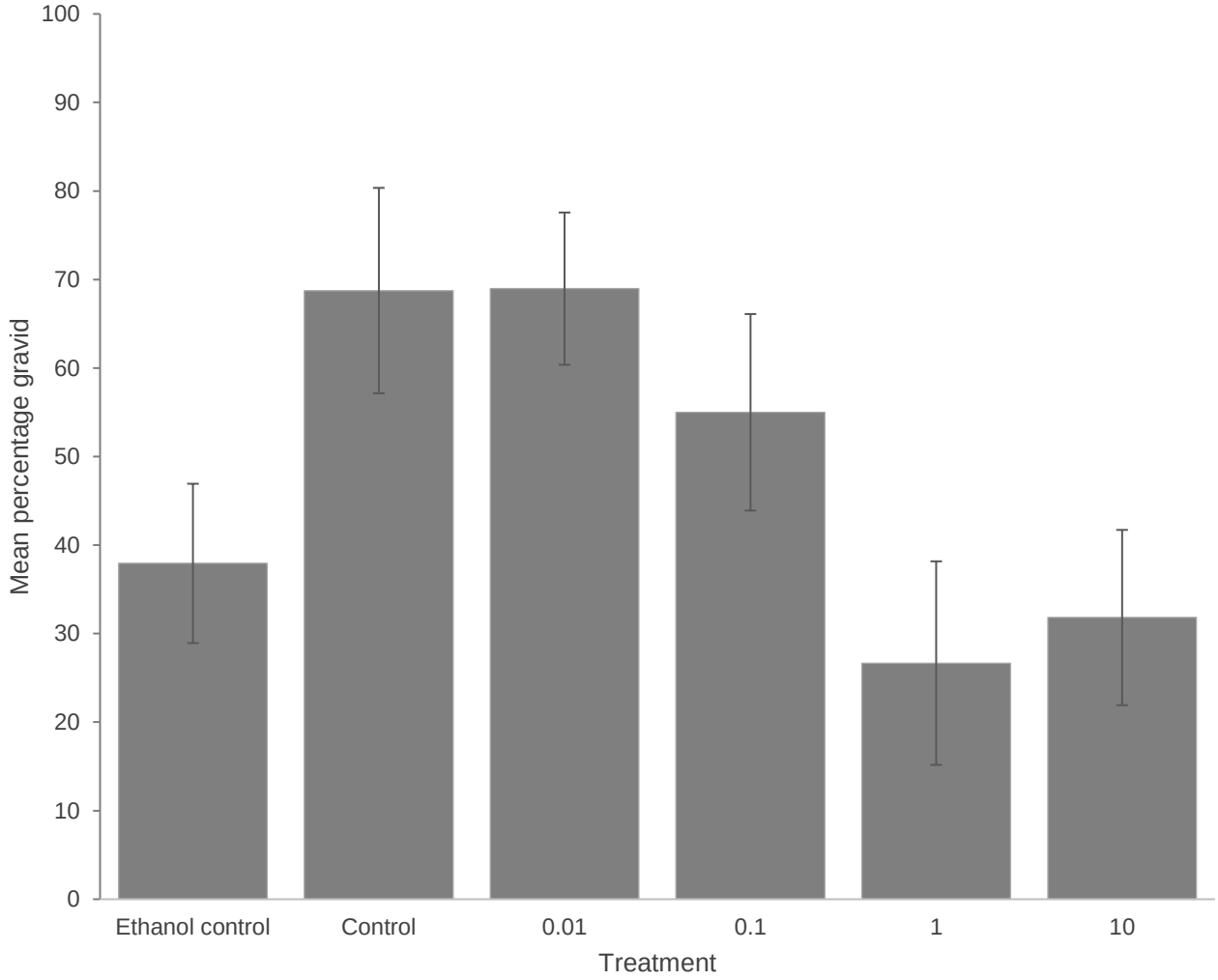

Figure 2.10 The mean (± SE) percentage of gravid *Aphodius prodromus* dung beetles after exposure over a period of 3 weeks to dung treated with ethanol only, water (control) or ivermectin at 0.01. 0.1, 1 or 10 ppm.

2.4 Discussion

2.41 Onthophagus similis

Lethal effects:

Ivermectin spiked into dung at a concentration of 1 ppm (ww) did not affect the mortality of mature adult *O. similis*. This concentration is comparable to the highest expected concentration excreted in dung when a pour-on formula of ivermectin is applied to cattle (Fig. 1.3). Some studies of paracoprid beetle support this conclusion. For example, Wardhaugh *et al.* (2001) found no significant difference in mortality of mature *O. taurus* or *E. fulvus*, in comparison to the control, when fed with dung from sheep fitted with ivermectin sustained-release boluses. Beetles tested in the latter study were sexually mature and collected from the field, as in the current work. The bolus dose per day was not specified, but a standard ivermectin bolus produces residues in dung of around 0.5 ppm (ww) for the whole period the bolus is active (Herd *et al*., 1996). The same result was found by Wardhaugh and Rodriguez-Menendez (1988) when sexually mature *B. bubalus* and *C. hispanus* were fed dung from cattle injected with 200 µg/kg ivermectin; no significant difference in mortality was observed.

In contrast, 80% adult mortality of *O. landolti* was observed after 10 days when adults were exposed to dung contaminated with 1 ppm (ww) of ivermectin (Perez-Cogollo *et al*. 2015). These beetles were relatively newly emerged, at 11 to 17 days old. High levels of mortality of the paracoprid beetle, *E. intermedius*, was also observed when exposed to dung containing ivermectin at 1 ppm; almost 50% of dung beetles died at this concentration (Cruz Rosales *et al.*, 2012). Similarly, these beetles were only 15 days old. A similar result was found when Wardhaugh *et al.* (2001) studied immature *O. taurus* beetles; newly emerged adults (2 days old) suffered mortality when exposed to residues of ivermectin from dung from sheep fitted with a sustained-release bolus. After 4 weeks they had suffered around 50% mortality, in comparison to the control group, which had suffered only 15% mortality. Wardhaugh and Rodriguez-Menendez (1988) observed 90% mortality of newly emerged *C. hispanus,* when using dung dropped 2/3 days after treatment of cattle with 200 µg/kg ivermectin. Substantial mortality was also recorded among

newly emerged beetles of *Onitis belial* (Fabricius) when fed dung from the same batch.

In conclusion, the data suggests that when dung beetles are mature, they may have greater resistance to ivermectin. In contrast, when dung beetles are newly emerged, they are likely to be more susceptible to ivermectin residues in dung. The current study is the first bioassay to use *O. similis* as a test organism. The age of the beetles used could not be determined, so in future, further studies with known newly-emerged *O. similis* beetles should be undertaken to see their response to ivermectin residues.

Sublethal effects:

Here, at environmentally relevant concentrations of ivermectin, sublethal effects on the reproductive system of *O. similis* were observed. Although not quantified, at higher concentrations of ivermectin the beetles appeared to have smaller fat bodies. The reduction in fat body size could be due to a decrease in feeding rate in the presence of ivermectin. Feeding activity was significantly suppressed when *C. hispanus* were fed dung from days 1 to 8 after injection of cattle with 200 µg/kg of ivermectin (Wardhaugh and Rodriguez-Menendez, 1988). The same was found for endocoprid beetle *A. sphacelatus* when exposed to dung spiked with 1 ppm ivermectin (ww); significantly less dung was consumed compared to the control group (Finnegan *et al.,* 1997).

Suppression of feeding may be caused by the paralysis of muscles, as a result of reduced frequency of action potentials in GABA-gated and glutamate-gated chloride channels (Zufall *et al.,* 1989). Verdu *et al.* (2015) found a significant relationship between muscle force of *Scarabaeus cicatricosus* (Lucas) and ivermectin concentration (0.001 to 0.2 ppm (ww)). At higher concentrations of ivermectin, muscle force was reduced. Indeed, in the current experiment dung beetles appeared slower, taking a longer time to react when removed weekly for counting. Muscles used by dung beetles to process food may also be affected. Therefore, at the concentrations used in the study (0.01 – 1 ppm), paralysis of the muscles of *O. similis* may have resulted in reduced feeding, and therefore, over the period of 3 weeks, a reduction in the size of the fat body. A reduction in motility would also affect mate and food-finding and reduce the ability of dung beetles to

escape from predators. A reduction in food uptake may also affect the reproductive physiology and output of female beetles.

Basal oocyte length of female *O. similis* was significantly smaller when beetles were exposed to dung spiked with ivermectin at 1 ppm (ww), compared to beetles exposed to dung spiked with lower ivermectin concentrations and the control group (Fig. 2.7; Fig. 2.8). A reduction in oocyte length was observed at all concentrations, although the difference was only significant at 1 ppm. A similar outcome has been demonstrated in other studies. In *E. intermedius* there was a relationship between oocyte size and ivermectin concentration, with the smallest basal oocytes recorded at the highest ivermectin concentration of 0.3 ppm (ww). Beetles used in the latter study were 7 to 15 days old (Martinez *et al*., 2017). In contrast, Cruz Rosales *et al*. (2011) found no effect of 1 ppm ivermectin treatment on the ovary physiology of 5 to 15-day old *E. intermedius*. Although, 50% mortality was observed and in the surviving beetles, fecundity was reduced. This study highlights that sublethal doses must be used for testing sublethal effects as high mortalities may leave a resistant population, skewing the results. Reduced oocyte size has also been demonstrated with exposure of beetles to doramectin and abamectin (Dadour *et al*., 2000). It is possible that no significant reduction in oocyte length was seen at concentrations below 1 ppm in the work described here, due to the maturity of the beetles.

The oocyte size reduction seen in the current study may have been due to a delay in development in response to ivermectin residues. Newly emerged *C. hispanus* showed delayed ovarian development when fed dung dropped 2/3 days after treatment of cattle with 200 µg/kg ivermectin (Wardhaugh & Rodriguez-Menendez, 1988). If the development of oocytes is delayed this can result in lower lifetime fecundity.

Reduced fecundity of paracoprid beetles in response to exposure to macrocyclic lactones has been reported in several studies. Kruger and Scholz (1997) found a reduction in the fecundity of 10-day old *E. intermedius,* using dung collected from 1 to 14 days after a 200 µg/kg injection of ivermectin to cattle. Significantly fewer brood masses oviposited by *E. intermedius* were also reported when they were exposed to dung spiked with 0.1 ppm of ivermectin (Cruz Rosales *et al.,* 2012). Beetles were between 5 and 15 days old. Brood ball production of *O. binodis* was

reduced by 67% using dung collected 1 week after cattle were treated by injection with avermectin B1 at 200 µg/kg (Ridsdill-Smith, 1988). Houlding *et al.* (1991) exposed *O. binodis* for 2 weeks to abamectin residues in bulked cattle dung collected 3 to 5 days after 200 µg/kg injection, and then for 6 weeks on clean dung. If beetles were 1 week old, a reduction in egg production of approximately 20% was found. If beetles were 8 weeks old, a reduction of 15% was found. If brood masses were counted in the current study, there may have been a similar outcome.

Alternatively, beetles may mature smaller oocytes in the presence of ivermectin. In this case, adult beetle size may be reduced. Insects of a smaller size are likely to have reduced survival, fecundity and mating success (Hirschberger, 1999; Lailvaux *et al*., 2005; Kingsolver & Huey, 2008; Reaney & Knell, 2015).

2.4.2 Aphodius prodromus

There was no clear pattern in mortality rates of *A. prodromus* in response to ivermectin, the statistically significant difference was primarily the result of the high mortality seen in the water control where 50% of individuals died within the first week. According to OECD guidelines if over 30% of individuals die in the control group then the test is not valid (OECD, 2010). Clearly the *A. prodromus* used in the current study did not cope well with the laboratory environment or some aspect of the procedure used. There was some evidence of an effect of ivermectin on the number of gravid females, a lower proportion of gravid females were seen in the higher ivermectin concentration treatments (Fig. 2.10), however, the ethanol control group had particularly low numbers of gravid individuals and, given the mortality rate, the value of these data is questionable.

2.4.3 Exposure to ML residues in the field

During the trials reported here, beetles were exposed to ivermectin at constant concentrations for 3 weeks. In reality, ivermectin degrades within a dung pat, becoming less toxic to insects, with a half-life of 1 to 6 weeks, depending on conditions (Halley *et al*., 1989; Sommer *et al.,* 1992). However, dung beetles do not remain in the same dung pat, preferring to colonise fresh dung. Therefore, the pattern of elimination of ivermectin from cattle must be considered. After pour-on treatment, ivermectin residues can be expected to peak at approximately 2 ppm (ww) on the first day after a 500 µg/kg pour-on application to cattle. The compound

is then rapidly eliminated, but is still detectable in dung dropped 28 to 35 days after application, at 0.002 ppm (ww). When ivermectin is administered via subcutaneous injection at 200 µg/kg to cattle, concentrations peak at 3 days after treatment at around 1 ppm (ww) and follow a similar route of elimination (Fig. 1.3). Therefore, dung beetles are likely to experience a range of concentrations of ivermectin, decreasing with time as the compound is eliminated from cattle. Movement between patches of differing residue concentrations should therefore also be taken in to account (da Silva & Hernandez, 2015). For example, in Finland *A. fossor* were found to take a median of 11 days to move between patches and *A. prodromus* moved after 7 days (Roslin, 2000). Movement will also be dependent on weather conditions, which control the moisture content of the dung pat. The movement of *Onthophagus* species may also depend on the soil type and its water content. *Onthophagus fracticornis* (Preyssler), *O. coenobita*, and *Onthophagus vacca* (L.) were found to take up to 8 days for 50% to emigrate when in optimum conditions (Sowig, 1995). In future experiments, when dung is replaced, it would be of interest to change the ivermectin concentrations to follow the pattern of elimination seen in the field.

One study attempted to replicate natural conditions found in the field. Bang *et al.* (2007) collected dung after cattle were sprayed with cis-cypermethrin and chlorpyrifos (2.1 g/cow). They fed *C. tripartitus* dung beetles a sequence of untreated dung, then dung 1, 3, 5 and 7 days after treatment of cattle. Dung was replaced in a 5-week cycle. The experiment found that the control group produced 30% more brood balls. The experiment could be improved by matching the day the dung was dropped with the number of days that have passed in the experiment. For example, when dung is replaced at the two-week mark, it should be replaced with dung dropped two weeks after treatment of cattle.

Beetles also move between patches at a pasture scale, which further complicates their probable pattern of exposure to endectocide residues. One study showed movement between pastures was more common in larger species of *Aphodius* (Roslin, 2000). During the experiment, which lasted 3 and a half weeks, one-third of *A. fossor* had moved between pastures. Dung beetles may move to pasture containing more recently treated cattle or to fields containing clean dung. Therefore, it is also important to study recovery of dung beetles in clean dung, as

well as continued exposure, as in the current study. One study showed that dung beetles appear to recover if given clean dung after exposure to contaminated dung (Manning *et al.*, 2017).

The current study used dung beetles collected from the field. These dung beetles would have represented a range of ages and reproductive states before they were taken in to the laboratory. There is also the possibility that they may have been exposed previously to endectocides, despite having been collected from an organic farm. Given a more extensive study, it would be preferable to breed an F1 generation of beetles in the laboratory in clean dung. This would ensure all dung beetles were of similar ages, unmated and unexposed to any endectocides. Fecundity could be measured directly by brood ball production. Additionally, the offspring could have been raised to adulthood to see if any transgenerational effects were present.

In this experiment the culture of *A. prodromus* failed. The conditions used in this experiment were clearly undesirable for this species. *Aphodius prodromus* emerge during early Summer, so the conditions used in this experiment may have been too warm (Gittings, 1994; Gittings & Giller, 1997). Additionally, *A. prodromus* breeds outside of dung, preferring to breed in decaying vegetation. If the experiment was repeated it should be conducted at a lower temperature and an appropriate substrate provided.

In future work, it may be preferable to test a greater number of concentrations. However, the present study was limited by the number of individuals caught in the week of trapping. If a culture could have been reared in the laboratory, this may have allowed for a larger sample size. Additionally, the presence of Clorsulon in the compound tested should have been included as a control, despite this material having any known insecticidal effect. It should also be considered that time of death may be a confounding factor; beetles which died earlier on in the experiment were exposed for a shorter amount of time to the treatment.

Overall, this study successfully demonstrated that the reproductive physiology of *O. similis* was affected by ivermectin. In the long-term, such effects may be ecologically damaging in terms of the abundance of dung beetles. The long-term effects of endectocides in the field were tested in Chapter 3.

Chapter 3 – The long-term effect of endectocide use on beef farms on dung beetle size and reproductive output

3.1 Introduction

Dung-colonising beetles are key members of farmland pasture systems, providing important ecosystem services (Beynon *et al*., 2015). Removal of dung and tunnelling by dung beetles reduces the number of cattle pests and parasites, whose lifecycle is associated with dung (Bornemissza, 1970; Sands *et al*., 2017). Additionally, their activity can improve soil structure and quality, which increases the biomass and nutritional value of pasture (Bang *et al.*, 2005; Yamada *et al.,* 2007). Lastly, degradation rate is increased by the presence of beetles, preventing dung from building up on pastures (Fincher, 1981).

However, in Northern temperate regions many dung beetle species are in widespread decline (Roslin *et al*., 2014; Natural England, 2016). Some of this decline can be attributed to an intensification of farming and habitat fragmentation (Hutton & Giller, 2003; Filgueiras *et al*., 2011). Many studies have also highlighted that endectocides applied to cattle, to control pests and parasites, are excreted in dung at high concentrations (Sommer *et al.*, 1992). Dung beetles are exposed to endectocide residues in dung, which exert lethal and sublethal effects (Wall & Strong, 1987; Kruger & Scholz, 1997; Perez-Cogollo *et al*., 2015).

Toxicology studies often focus on lethal effects of endectocides, despite the importance of sublethal effects on the physiology, behaviour and demographic of insect populations (de França *et al*., 2017). Studies of the sublethal effects of endectocides on dung beetles have reported reduced feeding rates, phenotypic malformations, extended development times and effects on reproductive physiology and fecundity (Finnegan *et al*., 1997; Kruger & Scholz, 1997; Dadour *et al*., 2000). Studies often focus on the toxicological effects of single endectocides in the laboratory over a short period of time. The sublethal effects demonstrated in the laboratory may not be representative of the field, so it is important to also study long-term effects.

The aim of this study, therefore, was to determine the effects of long-term use of macrocyclic lactones and synthetic pyrethroids in beef herds, on dung beetle size and reproductive physiology and output in the field. The hypothesis was that dung beetles from farms using macrocyclic lactones or synthetic pyrethroids would be smaller in size, and have a lower fecundity and smaller oocytes, than those from farms which did not use endectocides.

3.2 Methods

3.2.1 Farms

Beetles were caught as part of an earlier study by Dr Bryony Sands, from 24 farms in South-West England in 2016. When the initial study was designed, farms were selected on the basis that 12 were registered as organic and the other 12 were conventional farms. Organic and conventional farms were then paired according to proximity. However, once the study started, in reality, the categorisation of each farm was more complex. Cutting across the organic/conventional division, farms followed three different pest and parasite control regimes. Some used no endectocides at all (none) (n = 8), some used macrocyclic lactones only (MLs) (n = 9), the others used synthetic pyrethroids only (SPs) (n = 7). These practical groupings reflected the fact that some of the organic farms used SPs for pest control within the Soil Association regulations, while some of the farms that were not registered as organic did not use endectocides at all (Soil Association, 2018).

3.2.2 Trapping

Pitfall traps were set out in two blocks on pasture: early Summer (13th June – 26th July 2016) on all 24 farms, and late Summer (15th August – 8th September 2016) on 16 of the farms. Traps were set up between 9am and 12am and left for 24 hours. Trapping was carried out on paired farms on the same day to reduce variation.

10 traps were placed at each farm. The traps were set along a straight line transect, 5 m apart. The traps were separated from cattle by a fence to avoid damage, but still were within 50 m of a field grazed by cattle during the trapping period. One organic farm did not graze cattle in the required proximity to the pitfall traps, and so was removed from the study. Pitfall traps were of the same design as in Chapter

2 (Fig. 2.4; Fig. 2.5). Traps were baited with clean, homogenised dung from the farm of each pair designated as organic. The buckets were half-filled with water and 1 ml of detergent to kill beetles on capture. After collection beetles were preserved in ethanol in the dark, for later dissection.

3.2.3 Dissection

From the beetles collected by the pitfall traps, four species were selected for the study: *A. rufipes*, *A. fossor, O. coenobita* and *O. similis*. Beetles were identified using identification guides from Jessop (1986) and Skidmore (1991). Species were selected based on knowledge of their ovary physiology and oviposition behaviours, and on their relative abundance in the sample (Fig. 1.1; Fig. 1.2). Measurements were taken of the hind tibia, oocyte length and number of oocytes, as described in Chapter 2. The investigator was blinded to farm type until all dissections were complete. In total, 998 beetles were dissected.

3.2.4 Data analysis

The statistical package RStudio (Version 3.4.4) was used for data analysis.

Binary logistic regression:

Binary logistic regression was carried out with female reproductive state (defined as gravid or non-gravid) as the dependent variable, to determine whether season (early or late Summer) or farm type (ML, SP or none) could predict the reproductive state of a female. Again, individuals were defined as non-gravid if oocytes were undifferentiated, so that in *Onthophagus* species the coiled germarium was one mass or in *Aphodius* species the oocytes had not formed rounded ends. Binary logistic regression was used to handle the zero-inflated data as most of the females were non-gravid. Season was not included in the analysis for *O. coenobita* and *O. similis,* as no gravid individuals were found in the late season.

Two-way ANOVA:

Two-way ANOVAs were conducted to compare female beetle size, oocyte length and oocyte number in different farm types and seasons. Tukey post hoc tests were used to compare differences between groups. Where the distribution of the dependent variable was non-normal a Kruskal-Wallis test was conducted. This was the case for oocyte number and length for *A. fossor*. Here median values (±

interquartile range) are stated rather than means (± standard deviation). Only gravid females were included in the analysis of fecundity and oocyte length, but for analysis of size all females were included. The two *Onthophagus* species were not analysed for oocyte number and length due to small sample sizes. For all beetles, oocyte length and size of hind tibia were not correlated.

3.3 Results

3.3.1 Aphodius rufipes

For *A. rufipes*, 14% of the females had mature ovaries (n = 694). The mean number of oocytes found per gravid *A. rufipes* was 6.3 (±1.9) (n = 98). The mean length of a mature oocyte was 2.37mm (±0.18). The mean size of the female hind tibia was 2.17mm (±0.13).

Season was a significant predictor of the proportion of gravid *A. rufipes* (Table 3.1). A greater number of *A. rufipes* were gravid in late than the early Summer. In addition, there was almost a significant difference between seasons in oocyte number; individuals from the early season (5.4 (±1.9)) had fewer oocytes than those from the late season (6.5 (±1.9); Table 3.2). Season had no effect on oocyte length or size of female beetle (Tables 3.3, 3.4).

Farm type was not a significant predictor of the proportion of gravid females on farms and there was no difference in oocyte number between farm types (Table 3.1, 3.2). However, farm type did affect oocyte length (Table 3.3; Fig. 3.11A). There was no significant difference between farms using no endectocides and those that use MLs, but farms which use SPs (p = 0.01) had significantly larger oocytes than farms which omitted endectocide use. There was no significant difference between the two endectocide treatments. Additionally, size was significantly affected by farm type (Table 3.4; 3.11B). A one-way ANOVA showed individuals were significantly larger from farms using both MLs (t = 4.10; p < 0.001) and SPs (t = 2.00; p = 0.022) than from farms which used no endectocides. Beetles from farms which used MLs were also significantly larger in size than beetles from farms which used SPs (t = 2.01, p = 0.045).

3.3.2 Aphodius fossor

For *A. fossor*, 33% of the females were gravid (n = 55). The median number of oocytes found per gravid individual was 2 (±2) and the median length of a mature oocyte was 1.91 mm (±0.10) (n = 18). The mean size of beetles was 2.02 mm (±0.15).

Farm type was not a significant predictor of number of gravid females, and egg number or oocyte length did not differ between farm types (Tables 3.1, 3.2, 3.3). However, a significant difference was found in female size between farm types (Table 3.4; Fig. 3.12). A pair-wise comparison revealed this was between the two endectocide groups ($p < 0.001$). There was almost a significant difference between farms which use no endectocides and farms which use MLs ($p = 0.08$), but no significant difference was found between farms which use SPs and farms which do not use endectocides.

3.3.3 Onthophagus coenobita

For *O. coenobita*, 16% of the females were gravid (n = 104). The median number of oocytes found per gravid *O. coenobita* was 5 (±4.5) and the median basal length of oocyte was 1.51 mm (±0.86) (n = 17). The mean size of beetles was 1.70 mm (±0.14).

Farm type did not significantly affect the proportion of gravid females found on farms (Table 3.1). Season did affect size of *O. coenobita*; individuals caught in the early season were significantly larger than those caught in the late season (Table 3.4). Size was not significantly affected by farm type (Table 3.4).

3.3.4 Onthophagus similis

For *O. similis*, 6% of the females were gravid (n = 145). The median number of oocytes found per gravid female was 4 (±1.5) and the median basal length of oocyte was 1.44 mm (±0.13) (n = 9). The mean size of beetles was 1.38 mm (±0.16).

Farm type was a significant predictor of the number of gravid *O. similis* (Table 3.1; Fig. 3.13). A greater number of gravid individuals were found on farms where no endectocides are used. The highest proportion of gravid females was found at farms which forego the use of endectocides, then farms using SPs and finally those using MLs. There was a significant interaction between farm type and season on size of *O. similis* (Table 3.4; Fig. 3.14).

Table 3.1 The outputs from a binary logistic regression, where the effect of season (early or late Summer) and farm type (MLs, SPs or none) on reproductive state (gravid or non-gravid) of four UK dung beetles on farms in the South-West of England was examined.

Species	Comparison	Chi squared	*df*	*p*
A. rufipes	Interaction	3.85	4, 688	0.43
	Farm type	2.09	2, 690	0.35
	Season	55.11	1, 693	<0.001***
A. fossor	Farm type	0.03	2, 54	0.99
O. coenobita	Farm type	3.64	2, 25	0.16
O. similis	Farm type	15.42	2, 63	<0.001***

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 3.2 The outputs from an ANOVA, where the effect of season (early or late Summer) and farm type (MLs, SPs or none) on oocyte number of two UK dung beetle species during Summer on farms in South-West England was examined.

Species	Comparison	F/H	*df*	*p*
A. rufipes	Interaction	2.16	2, 92	0.12
	Farm type	2.17	2, 92	0.12
	Season	3.76	1, 92	0.06
A. fossor	Farm type	0.25	2, 15	0.88

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 3.3 The outputs from an ANOVA, where the effect of season (early or late Summer) and farm type (MLs, SPs or none) on oocyte length of two UK dung beetle species during Summer on farms in South-West England was examined.

Species	Comparison	F/H	*df*	*p*
A. rufipes	Interaction	2.22	2, 92	0.11
	Farm type	3.60	2, 92	0.031*
	Season	0.28	1, 92	0.60
A. fossor	Farm type	2.07	2, 15	0.36

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 3.4 The outputs from an ANOVA, where the effect of season (early or late Summer) and farm type (MLs, SPs or none) on body size of four UK dung beetle species during Summer on farms in South-West England was examined.

Species	Comparison	F	*df*	*p*
A. rufipes	Interaction	0.82	2, 688	0.44
	Farm type	8.09	2, 688	<0.001***
	Season	1.31	1, 688	0.25
A. fossor	Farm type	4.17	2, 52	0.021*
O. coenobita	Interaction	1.10	2, 98	0.34
	Farm type	1.54	2, 98	0.22
	Season	13.47	2, 98	<0.001***
O. similis	Interaction	6.02	2, 139	0.003**
	Farm type	51.00	2, 139	<0.001***
	Season	0.05	2, 139	0.82

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

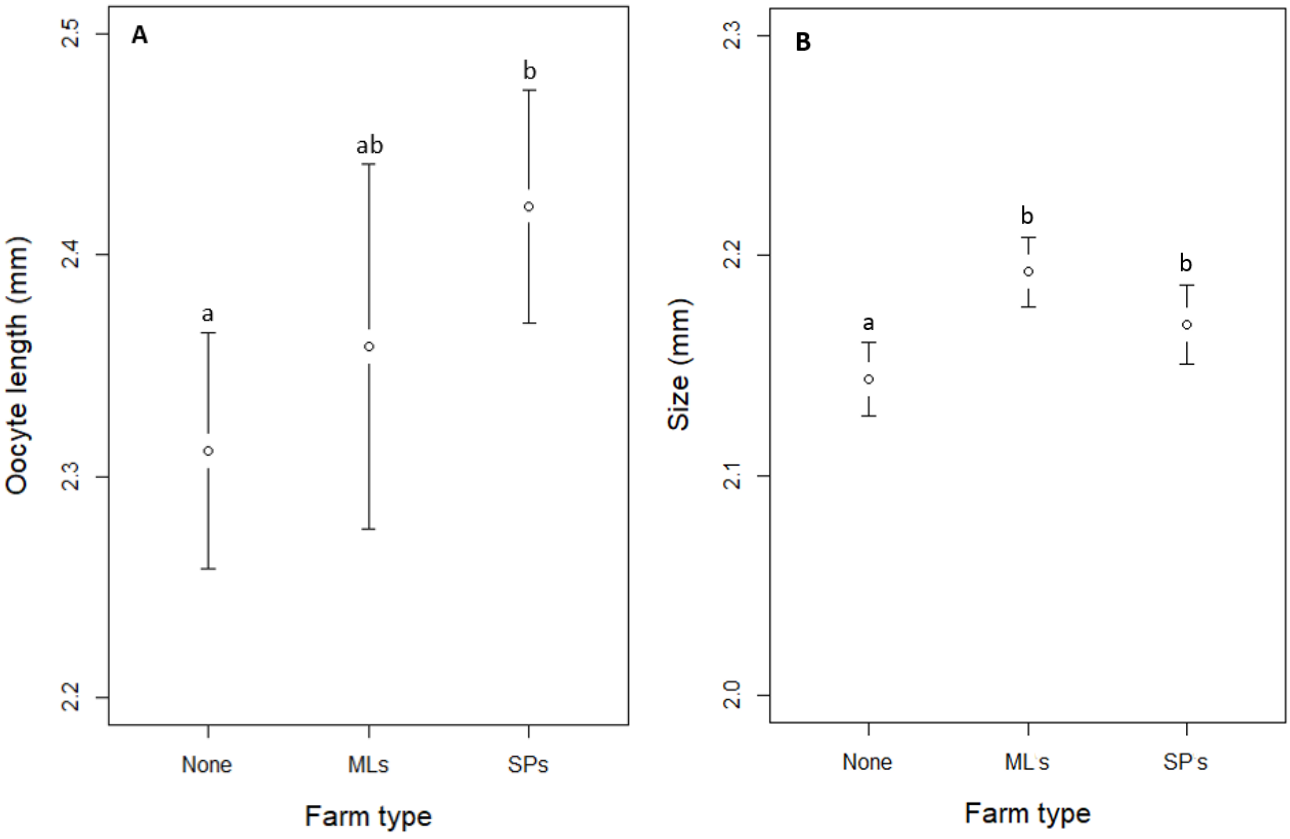

Figure 3.11 Effect of farm type (none, macrocyclic lactones, synthetic pyrethroids) on A. oocyte length and B. size of *Aphodius rufipes* dung beetles during Summer on farms in South-West England. Means are displayed with ±95% confidence intervals and letters above bars denote significant differences between means.

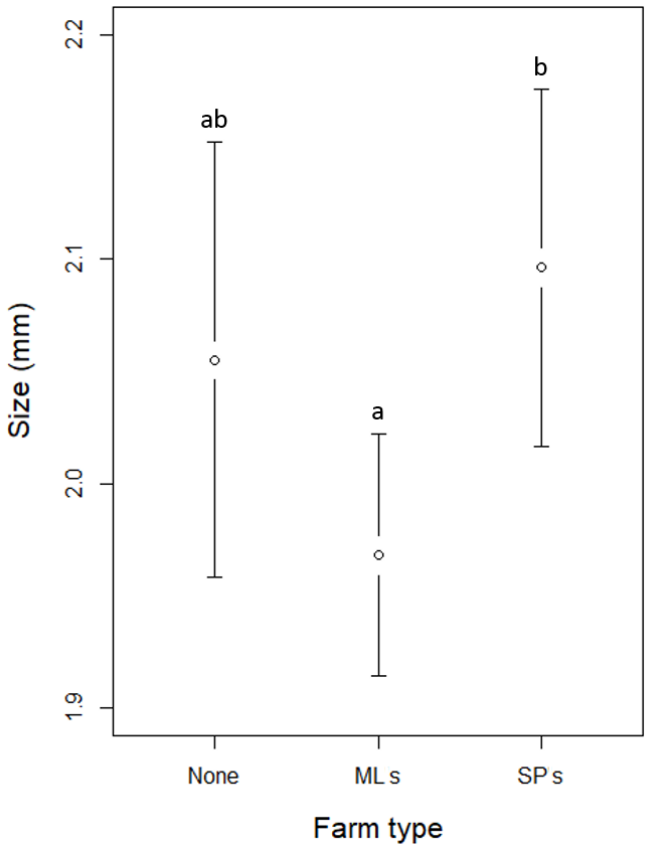

Figure 3.12 Effect of farm type (none, macrocyclic lactones, synthetic pyrethroids) on size of *Aphodius fossor* dung beetles during Summer on farms in South-West England. Means are displayed with ±95% confidence intervals and letters above bars denote significant differences between means.

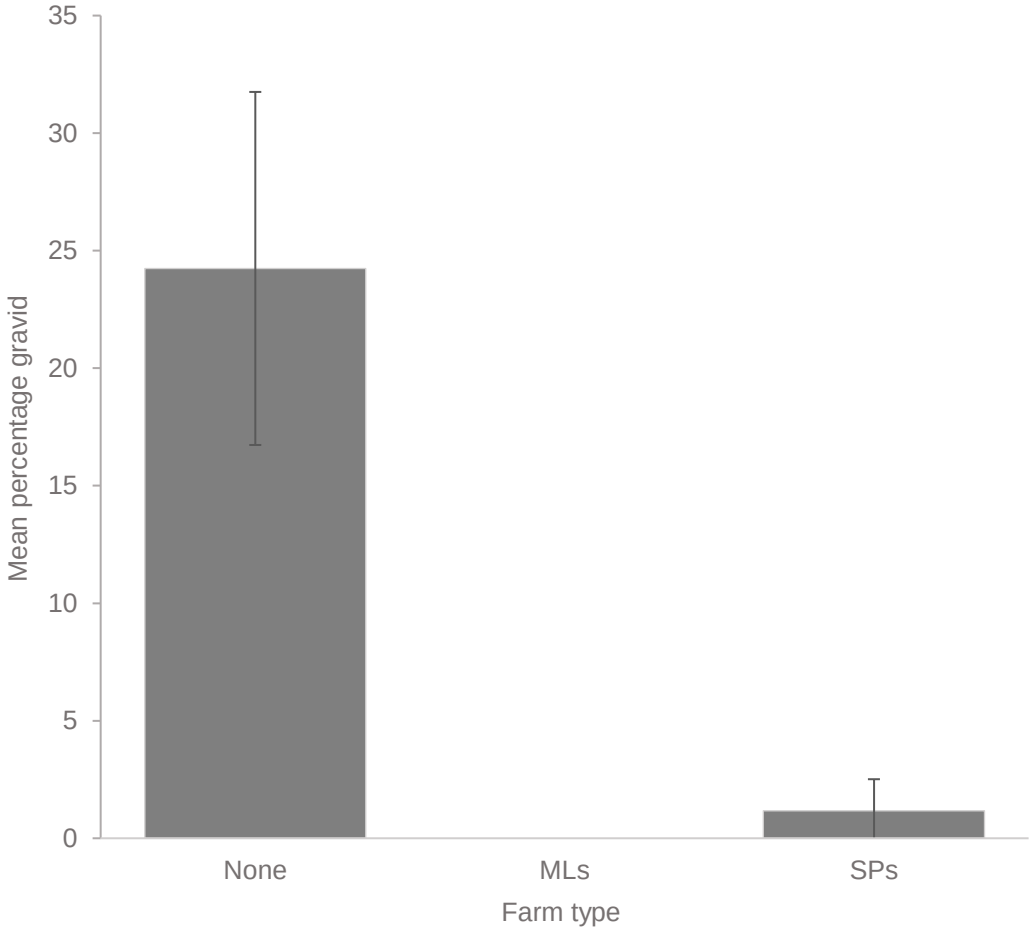

Figure 3.13 Proportion (%) of gravid *Onthophagus similis* dung beetles during Summer on different farm types (none, macrocyclic lactones, synthetic pyrethroids) in South-West England. Bars display the standard error.

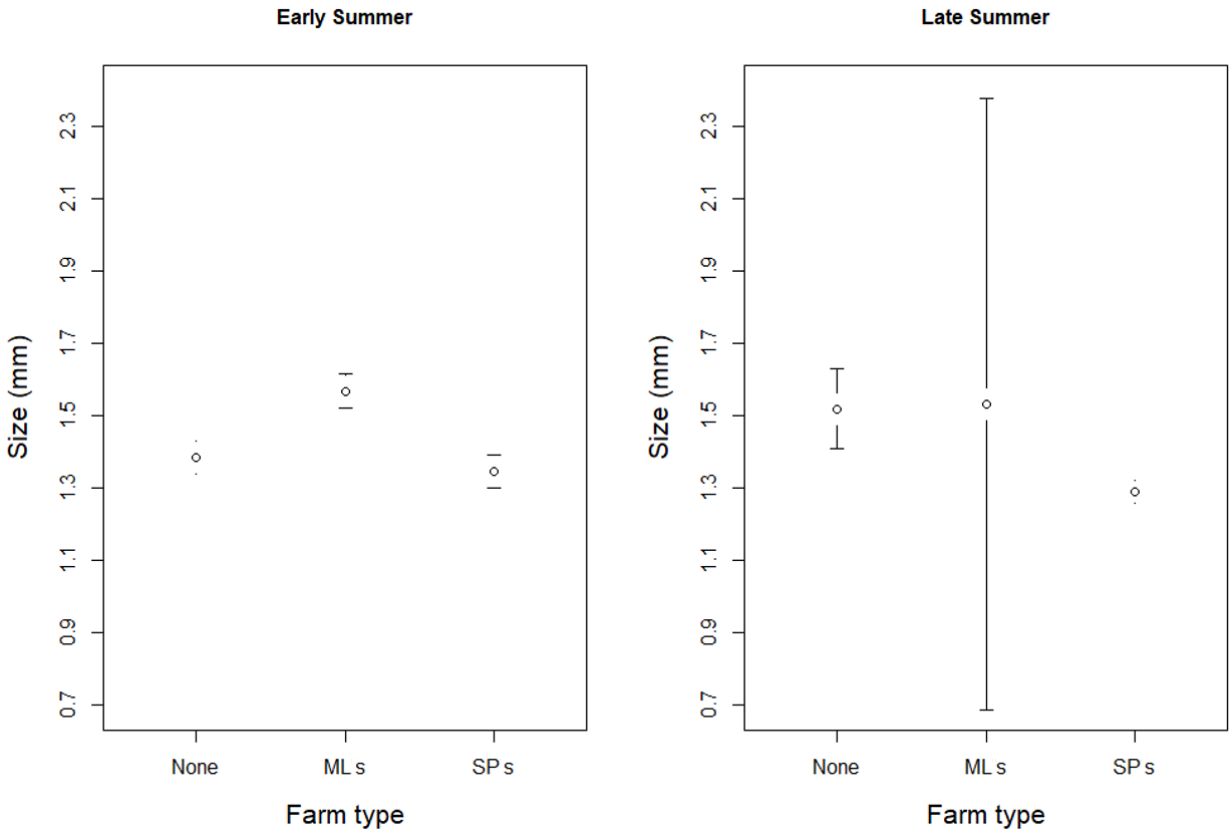

Figure 3.14 Interaction between farm type (none, macrocyclic lactones, synthetic pyrethroids) and season on size of *Onthophagus similis* dung beetles on farms in South-West England. Bars display ±95% confidence intervals.

3.4 Discussion

3.4.1 Life history

This study shows that, at any one time, only a relatively small proportion of females had mature ovaries. This was true for all four species included in this study, from both genera.

Aphodius rufipes overwinters mainly at the final larval stage, with a small number of individuals overwintering as adults. The adult flight period begins in mid-May and lasts until early September. Therefore, individuals from a large range of ages and reproductive states are likely to be present during the whole Summer season (Gittings, 1994; Gittings & Giller, 1997). The combination of overwintering at different life stages, and lack of synchrony in emergence, may result in only a small proportion of sexually mature beetles at one time, and therefore few females with mature ovaries. Gittings and Giller (1997) also found more gravid *A. rufipes* in the late Summer, in accordance with the present study.

Aphodius fossor overwinters mostly as an adult and their flight period begins earlier than *A. rufipes*, at the start of May. The lack of individuals with mature ovaries in this study may also indicate a lack of synchrony in emergence. Gittings and Giller (1997) found individuals at a range of reproductive states present for the whole season but did not record age. Additionally, in accordance with this study, they found few *A. fossor* in the late season; a total of 6 females during July.

The average number of mature oocytes found in this study for *Aphodius* beetles corresponds well with other reports. Gittings and Giller (1997) found an average of 8 oocytes produced by *A. rufipes,* and an average of 2 oocytes produced by *A. fossor*. This study recorded an average of 6 and 2 mature oocytes, respectively. The large difference in oocyte number between *A. rufipes* and *A. fossor* is due to the difference in their ovariole development. *Aphodius rufipes* develop batches of oocytes simultaneously. In contrast, *A. fossor* undergoes sequential development so only a few oocytes are matured at a time.

The very low numbers of gravid *Onthophagus* females may also suggest that only a relatively small proportion of individuals are sexual mature at one time. However, no studies have yet been completed on the synchrony of emergence of either

Onthophagus species in the present study, or on their overwintering stage. Studies on other species of *Onthophagus* show that most individuals overwinter at the adult stage, with fewer individuals overwintering as larvae (Hunter *et al*., 1991; Yasuda, 1991; Tyndall-Biscoe & Walker, 1992; Hunter *et al*., 1996). This is the first study to record the reproductive physiology of *O. similis* and *O. coenobita*.

3.4.2 Sublethal effects

Aphodius rufipes appeared to benefit from the use of endectocides; oocytes were significantly larger on farms which used SPs, and on farms which used MLs, although this was not significant at $p < 0.05$. Additionally, female beetle body size was larger from farms that used MLs and SPs than those which did not use endectocides.

Previous work, with the same sample of beetles as used in the present study, showed that there were no differences in overall beetle abundance between farm types, however species richness was approximately 34% higher on farms with a history of using no endectocides compared to farms that used endectocides (Sands & Wall, in press). The study found that *Aphodius* species dominated the community on farms that used endectocides, particularly MLs, while paracoprids such as *Onthophagus* were rare, possibly due to differential sensitivity of the functional groups. *Aphodius rufipes* was particularly abundant on the farms which used endectocides. They suggested that *Aphodius* may be more resistant than the other species to endectocides and removal of more sensitive competing dung-beetle species may allow the resource to be dominated by *Aphodius* (Sands & Wall, in press). If *A. rufipes* was benefiting from a reduction in competition from other species, this could result in a larger size of *A. rufipes* dung beetles seen here. Size and egg size often vary allometrically in insects, meaning larger individuals usually have larger eggs (Kingsolver & Huey, 2008).

Due to their oviposition behaviour, there is potential for competition between *Aphodius* dung beetles, at the adult or the larval stage (Hanski & Cambefort, 1991). However, competition can only occur if a resource is in limiting supply. Overall, at natural densities, a maximum of 0.2% of the energy found in a dung pat is consumed by a mixed assemblage of *Aphodius* dung beetles (Holter, 1982). Up to 50% of the particles in dung are small enough to be consumed by *Aphodius* dung

beetles, but this still leaves an excess of dung for consumption (Holter, 2000). The same is the case for larvae; calculations show at natural densities competition for food does not occur (Finn & Gittings, 2003). Occasionally, *Aphodius* dung beetles can be found at densities of 1000s of beetles per dung pat (Hanski, 1979; Holter, 1982). Competition for food is probable at these densities but they are rarely recorded in studies.

Competition for space at natural densities between *Aphodius* dung beetles is more probable, particularly at the larval stage. Larvae need a large amount of space in comparison to their body volume for development. Additionally, unlike adults, they are not able to move to another dung pat to reduce population density. Landin (1961) calculated that third-stage larvae need a space of up to 150 times their own volume. This makes competition between *Aphodius* dung beetles at natural densities likely.

Paracoprid beetles are more likely to exert a competitive pressure on *Aphodius* dung beetles, as they remove large quantities of dung below ground as brood balls. This reduces the food availability for *Aphodius,* as well as space. In addition, *Geotrupes* (up to 26 mm in length) species are much larger than *A. rufipes* (9 – 13 mm in length) and so can process a greater quantity of dung (Horgan, 2001). Gittings and Giller (1999) found rapid decomposition of dung pats occurred in late Summer in Ireland, probably preventing the development of any *Aphodius* larvae. This was associated with the presence of *Geotrupes spiniger* (Marsham) in combination with weather conditions. The greater quantity of dung on the pasture, due to absence of *Geotrupes*, may create a lower larval density of *A. rufipes*. Gittings (1994) found that at lower larval densities of *Aphodius ater* (De Geer), the mean size of third-instar larvae was larger. Therefore, the reduction in abundance of *Geotrupes* and *Onthophagus* on farms where endectocides are used could cause the abundance, body size and oocyte size of *A. rufipes* to increase.

In contrast, *A. fossor* were smaller on farms using MLs and *O. similis* showed a greater variation in size when exposed to MLs during the late Summer. Dung beetles are holometabolous, so adult size is determined by larval feeding. If larval feeding is impaired by endectocides, the growth of larvae could be stunted, resulting in smaller adult beetles. Larval nutrition has also been shown to indirectly effect adult weight gain (Reaney & Knell, 2015).

A reduction in size of larvae in the presence of MLs has been demonstrated in the laboratory. For example, Cruz Rosales *et al*. (2011) found the width of the cephalic capsule at the third instar larval stage of *E. intermedius* was reduced at concentrations of 0.01 ppm (ww) of ivermectin. Sommer & Nielsen (1992) also found a reduction in larval mandibular and clypeal size when exposed to dung collected 17 days after cattle were injected with a dose of 200 µg/kg of ivermectin. Additionally, adult body size of *E. intermedius* decreased in females and major males at concentrations of 0.003 to 0.5 ppm (ww) ivermectin (Gonzalez-Tokman *et al*., 2017).

The paralysing effect of endectocides may reduce feeding in larvae and therefore larval size. Reduced feeding has been reported in adult *Aphodius* dung beetles when exposed to 1 ppm ivermectin (ww) (Finnegan *et al*., 1997). Additionally, pharyngeal pumping is inhibited in the nematode worms *Ascaris suum* and *Haemonchus contortus,* preventing feeding, down to 0.1 nM of ivermectin (Geary *et al*., 1993; Brownlee *et al*., 1997). As muscles are used during larval feeding, it is probable that feeding is also impaired by endectocides at the larval stage.

Being small has negative consequences for fitness. This relationship has been shown to be common across invertebrates. A meta-analysis of 52 papers on the effect of size on fitness in invertebrates showed that larger individuals had greater survival in 76% of cases, greater fecundity in 85% of cases and greater mating success in 74% of cases (Kingsolver & Huey, 2008).

The fitness benefits of a larger size have also been demonstrated in dung beetles specifically. One study found size and horn size are the main predictors of the outcome of competition between males, with horn size increasing in importance as males became larger (Pomfret & Knell, 2005). Another study found horn size predicted how far *E. intermedius* can run before becoming exhausted (Lailvaux *et al*., 2005). Horn size is also determined by post-eclosion larval feeding and is fixed during adulthood (Reaney & Knell, 2015). Female dung beetles also suffer a reduction in fitness when size is reduced. Fecundity of *A. ater* was found to be positively related to female size (Hirschberger, 1999).

The reproduction of *O. similis* appeared to be negatively affected by use of endectocides; farms using MLs and SPs had virtually no gravid females, although,

for the females that were gravid, oocyte size and number were not affected. Many studies have found reduced fecundity or reduced brood ball production when paracoprid species are exposed to MLs or SPs at environmentally relevant concentrations, as previously mentioned (Kruger & Scholz, 1997; Vale *et al*., 2004; Cruz Rosales *et al.*, 2012; Martinez *et al*., 2017; Sands *et al.*, 2018). Endectocides could have direct effects on reproduction, delaying oocyte maturation or reducing fecundity. Alternatively, the mechanism could be indirect; muscle paralysis may result in reduced feeding or ability to find a mate. A recent study has shown that ivermectin affects the detection of volatiles by the antennae. A reduction in detection occurred at all concentrations tested (0.001 to 0.2 ppm). Dung patches are highly ephemeral so if volatile sensitivity is altered this could have drastic effects on food and mate finding.

Sublethal toxicity studies on paracoprid beetles largely focus on tropical species and do not look at the long-term effect of endectocides in the field. It is important to study the long-term effects in natural conditions as results could differ to those found in short-term laboratory studies. This study is the first to find potential long-term reproductive consequences of endectocide use in the field on temperate paracoprid beetles.

3.4.3 Improvements

The current work is an observational study, therefore there are confounding factors which may have affected the results. Despite efforts to reduce variation by pairing farms according to proximity, farms varied in aspects such as substance used for treatment, frequency of treatment, proportion of herd treated and the period of time since treatment. These factors would have altered how toxic the dung was to dung beetles. The breed of cattle and terrain type also differed between sites. This may have altered the attractiveness of the sites to dung beetles, which could cause variation in the species present on the site.

Chapter 4 – Consequences of sublethal effects to dung beetles on pasture ecology

4.1 Consequences to dung beetle abundance and ecosystem services

Dung-dwelling beetles play an important role in pasture systems by providing services which maintain ecosystem function. In the U.K., the value of these services is estimated at 367 million pounds per annum (Beynon *et al*., 2015). Additionally, dung beetles are part of a complex web of interactions with other species in pasture systems, thought to provide food for up to 409 species of birds, mammals, reptiles and amphibians globally (Young, 2015). However, the sublethal effects of endectocides at the egg, larval or immature adult stage, combined with lethal effects, are likely to reduce the long-term abundance of dung-beetles on farms using endectocide treatments.

Models, parametrized with laboratory data, have been used to calculate how the abundance of dung beetles might be affected in pasture systems under endectocide treatment. One study modelled the lethal effects of ivermectin on *Aphodius* dung beetle larvae (Sherratt *et al.*, 1998). Under a standard anthelmintic regime in northern Europe, a decrease in population size of approximately 10% for each application of ivermectin was predicted. This equated to around a 25% reduction over the whole season. Another study modelled immature *O. taurus* survival in the presence of eprinomectin or moxidectin (Wardhaugh *et al.*, 2001). This paracoprid species was once present in the UK but is now extinct nation-wide. Moxidectin was predicted to have no effect on population abundance, but eprinomectin was shown to reduce abundance of the dung beetle. It was predicted that the abundance of this multivoltine (several generations per year) species would suffer more than univoltine species. This could be why paracoprid *O. similis* appeared to be more susceptible to long-term endectocide treatment than the other beetle species. The study suggested that a single treatment of eprinomectin would reduce abundance of *O. taurus* by 35% when cattle were treated 14 days after emergence of beetles. This is a time where a large number of immature beetles would be exposed, which show a greater susceptibility than mature adult beetles to endectocide residues.

Neither of these modelling studies took in to account the sublethal effects of endectocides on reproductive output. The addition of sublethal effects to models is likely to indicate that there would be further reductions in abundance of certain species. These studies also only looked at single species or genera at one life-cycle stage. Age-specific mortality should be included in models as larvae and immature adults suffer greater mortality than mature adults (Wardhaugh & Rodriguez-Menendez, 1988; Wardhaugh *et al.*, 2001). Species interactions are also likely to be important in determining changes in species' abundance in response to endectocides. In this study, *A. rufipes* appeared to benefit from the use of endectocides. Not only was the species larger with larger eggs, but it was also greater in abundance on farms which used MLs and SPs (Sands & Wall, in press). As discussed previously (Chapter 3), this may be due to a reduction in competition from other species which are more susceptible to endectocides, resulting in a higher abundance of *A. rufipes*. Current models could be improved by including interactions between different species, although this would be complex.

The reduction in abundance of certain species and resultant change in community structure is likely to have a functional impact and affect the ecosystem services provided by dung beetles. Studies assessing the contribution of different species have demonstrated differential contributions of individual species. For example, Rosenlew & Roslin (2008) found that large paracoprid *Geotrupes stercorarius* (L.) contributed disproportionately to changes in fresh dung weight; when the species was added the amount of dung was removed was doubled. Overall, capacity to remove dung also appears to increase with size. Examination of the effect of eight dung beetle species, half paracoprid and half endocoprid, showed that dung beetles with a greater body mass removed more dung (Nervo *et al*., 2014).

There is considerable evidence that more complex beetle communities achieve the greatest differences in ecosystem services. Over a period of 36 weeks, mixed species assemblages of *Aphodius* and *Onthophagus* dung beetles were shown to be able to achieve the highest decomposition rates in comparison to monoculture treatments, when the biomass of beetles was standardised (Beynon *et al.*, 2012). A similar conclusion was reached in a study by Manning *et al.* (2017) where the dung-removing capacity of *A. ater*, *A. fossor* and *O. joannae* was assessed in polycultures or monocultures. The three species treatment and the monoculture containing *A.*

fossor removed a larger quantity of dung than the other two treatments. A mixed community including the paracoprid beetle *O. nuchucornis* was also found to be able to remove a greater quantity of dung than the endocoprids, *A. erraticus* and *A. fossor* in monocultures (Manning & Cutler, 2018). Changes in gas fluxes under different beetle treatments have also been assessed. Mixed dung beetle assemblages were shown to result in 32% lower CO_2 equivalent emissions compared to monoculture treatments (Piccini *et al.*, 2018).

Overall, therefore, studies highlight that species are not functionally redundant; the greater abundance *A. rufipes* on farms using endectocides is unlikely to replace the ecosystem services provided when a species rich assemblage is present. The reduced abundance of *O. similis* on farms, perhaps due to sublethal effects on reproduction, may have disproportionate effects on the ecosystem services provided by dung beetles (Sands & Wall, in press). Additionally, having multiple species buffers the system from perturbation. Hence, species richness in pasture decomposer systems would appear to be of value and should be conserved.

Climate change could exacerbate the problems caused by endectocides. Using warming chambers, a study experimentally increased temperature by up to 5 °C and examined the ability of different northern temperate dung beetles to increase plant productivity (Slade & Roslin, 2016). Plant productivity was measured in terms of ryegrass biomass, grown inside the chambers. In control groups dung beetles increased plant productivity by mixing the soil with dung. However, in heated chambers the effects of dung beetle monocultures on plant productivity decreased. Differences were evident when the temperature was only 1 °C warmer. This effect was reduced when both paracoprid (*G. stercorarius* and *G. stercorosus*) and endocoprid (*A. fossor*) beetles were present in a polyculture. This again highlights the importance of maintaining species richness. Artificial warming by 2.3 °C advanced egg-laying and decreased the larval size of *A. erraticus* by 33.4% (Wu & Sun, 2012). The current study also showed a decrease in size of *A. fossor* and an increase in variation of size in *O. similis* in the presence of endectocides. Increased temperatures due to climate change could further decrease size of beetles, resulting in reduced fitness. Additionally, changes in phenology coupled with the likely delays in reproduction, may alter the seasonal synchrony of species.

4.2 Minimising the effect of endectocides on dung beetles

This study has shown that the reproductive physiology and output of some dung beetle species is negatively impacted by endectocides and suggests that this may result in a reduced abundance of these species. However, as discussed in Chapter 1, the use of endectocides in cattle farming is considered to be essential by many farmers for maintaining high yields. If endectocides were banned, considerable economic loss and a reduced national capacity to provide livestock-based food might result. To prevent harm to dung beetles, farmers can instead make changes to their cattle treatment regimes.

In the models discussed above, the factors which affected dung beetle abundance the most were: the length of time the endectocide was active, the number of cattle treated, how long the dung was attractive to beetles, and when endectocides were applied to cattle (Sherratt *et al*., 1998; Wardhaugh *et al.*, 2001).

There are endectocides available on the market which are less toxic to dung beetles, due to their shorter period of activity. Of the macrocyclic lactones, several studies have found that the most toxic compound is doramectin, followed by ivermectin, eprinomectin and moxidectin (Strong & Wall, 1994; Dadour *et al*., 2000; Floate *et al*., 2001, 2002). Moxidectin has been shown to be 64 times less toxic to fly and beetle larvae than abamectin (Doherty *et al*., 1994). For synthetic pyrethroids, there are fewer studies that make direct comparisons, but no difference in beetle mortality after exposure to deltamethrin, cypermethrin and cyfluthrin were reported by Sommer *et al*. (2001), although flumethrin was found to be less harmful than the other compounds tested. However, Bianchin *et al.* (1998) found no significant difference in mortality between all these products.

Differences in toxicity also occur between different endectocide administration routes. Overall, compounds applied by pour-on treatment appear to be found at higher concentrations in the dung and therefore exert stronger effects on dung beetles than injection and spray on treatments (Sommer *et al.*, 1992; Bianchin *et al.*, 1997; Vale *et al.*, 2004). Boluses are active for a long time, so residues are present in dung for extended periods, with a resulting prolonged effect on fauna.

Alternative substances, which may be less harmful to dung beetles due to their selectivity, are available on the market. For example, essential oils, biocontrol via

parasitoids, fungi, viruses and trapping (Broughan & Wall, 2005; Briggs *et al*., 2006; Geden & Hogsette, 2006; Ellse *et al*., 2013). However, currently they have relatively low efficacy against pests and parasites, compared to the synthetic endectocides, or are too expensive to produce commercially. For example, essential oils are extremely volatile, so are presently not sufficiently effective and are expensive to produce. These products may become more important in the future due to growing level of resistance seen in many pests and parasites to synthetic endectocides. Resistance to anthelmintics has been reported extensively in nematodes of cattle (Waghorn *et al*., 2006; Sutherland & Leathwick, 2011).

In the UK, most cattle are treated at Spring turnout, when they are moved onto pasture. This coincides with the peak time of emergence for the majority of dung beetles. At this time, dung beetles are immature are so are at their most vulnerable to endectocide residues in dung. Earlier treatment, while cattle are still in house, or a delay in treatment, to allow beetles to mature when they have greater resistance may be beneficial. Although, this would have production consequences since it would be less efficient at controlling parasitic nematodes. It would be also beneficial to only treat cattle when necessary. For example, faecal egg counts (FEC) could be used to diagnose individuals with high parasite loads and only these individuals could be treated. This would increase the amount of clean dung available to dung beetles on pasture and may also help to reduce the rate of development of resistance of parasites to endectocides.

Negative effects of endectocides on the reproductive physiology and output of dung beetles may be equally as detrimental as lethal effects. Future studies should continue investigation of their impacts on reproduction, with an emphasis on temperate species, where the literature is sparse. Additionally, further testing of the long-term effect of endectocides in pasture systems should be undertaken. The adverse effects of endectocide treatment on dung beetles could be mitigated by reducing treatment to only parasitized cattle, using compounds such as moxidectin, which has a lower toxicity to dung beetles, and by administering treatment by injection to reduce the residue concentrations found in dung. A change in timing of cattle treatment should also be considered to reduce exposure of immature beetles to residues.

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